# Desert Pupfish
***(Cyprinodon macularius)***

# 5-Year Review:
# Summary and Evaluation

**U.S. Fish and Wildlife Service**
**Arizona Ecological Services Office**
**Phoenix, Arizona**

**2010**

# 5-YEAR REVIEW
# Desert pupfish/*Cyprinodon macularius*

## 1.0 GENERAL INFORMATION

### 1.1 Reviewers

| | |
|---|---|
| **Lead Regional Office:** | Southwest Regional Office, Region 2<br>Susan Jacobsen, Chief, Threatened and Endangered Species, 505-248-6641<br>Wendy Brown, Recovery Coordinator, 505-248-6664 |
| **Lead Field Office:** | Arizona Ecological Services Field Office, Phoenix<br>Steve Spangle, Field Supervisor, 602-242-0210, Extension 244 |
| **Cooperating Field Offices:** | Arizona Fish and Wildlife Conservation Office<br>Jeremy Voeltz, Fish Biologist, 928-338-4288<br><br>Carlsbad Fish and Wildlife Office<br>Carol Roberts, Division Chief/Salton Sea Coordinator, 760-431-9440, Extension 271 |
| **Cooperating Regional Office:** | Pacific Southwest Regional Office, Region 8<br>Larry Rabin, Deputy Division Chief for Listing, Recovery, and Habitat Conservation Planning, 916-414-6464 |

### 1.2 Methodology used to complete the review:

This review was completed by the Tucson Sub-office of the Arizona Ecological Services Office of the U.S. Fish and Wildlife Service (USFWS). In addition to the general solicitation of public comments published in the Federal Register (71 FR 20714), we sent a specific request for new information related to conservation and natural history of the desert pupfish (*Cyprinodon macularius*) (or the subspecies *C. m. macularius* or *C. m. eremus*) to a number of individuals with a history of working on desert pupfish research and conservation (see REFERENCES section).

Recommendations resulting from this review are a product of thoroughly reviewing all available information on the desert pupfish. We reviewed past and recent literature, public comments, the listing rule, and the recovery plan (USFWS 1993). Interviews with individuals were conducted as needed to clarify or obtain specific information. Outreach consisted of a Federal Register Notice (71 FR 20714) that requested any new information about the desert pupfish related to population trends, distribution, habitat conditions, threats, taxonomy, and conservation measures from the public, concerned governmental agencies, Tribes, the scientific community, industry, non-profit conservation organizations, and any other interested parties. Data and additional information was received from the Arizona Game and Fish Department (AGFD) (AGFD 2006) and Organ Pipe Cactus National Monument (USNPS 2006). We attempted to get information from Mexico, but were not successful. This review reflects our current state of knowledge regarding the status of desert pupfish. Definitions for wild or captive populations, Tier 1, 2, and 3 populations, and viable populations are all found in the recovery plan (USFWS 1993), and described on page 6 of this document and under Recovery Criterion 2.

### 1.3 Background:

The desert pupfish is a small fish, less than three inches long, and a member of the Cyprinodontidae family (Minckley 1973). The body is thickened and laterally compressed; coloration is a silvery background with narrow dark vertical bars on the sides. The protruding mouth is equipped with tricuspid teeth and the desert pupfish has an opportunistic, omnivorous diet, consisting of invertebrates, plants, algae, and detritus (Cox 1966 and 1972; Naiman 1979). Males are larger than females and become bright blue with orange-tipped fins during the breeding season and exhibit aggressive, territorial behavior (USFWS 1993). Spawning occurs from spring through autumn, but reproduction may occur year-round depending on conditions (Constanz 1981). The desert pupfish appears to go through cycles of expansion and contraction in response to natural weather patterns (USFWS 1986, 1993; Weedman and Young 1997). In very wet years, populations can rapidly expand into new habitats (Hendrickson and Varela-Romero 1989). In historical times, this scenario would have led to panmixia among populations over a very large geographic area (USFWS 1993).

The desert pupfish has a tolerance for high temperatures, high salinities, and low dissolved oxygen concentrations that exceed the levels known for other freshwater fishes (USFWS 1993). Habitats have included clear, shallow waters with soft substrates associated with cienegas, springs, streams, margins of larger lakes and rivers, shoreline pools, and irrigation drains and ditches below 1,585 meters (5,200 feet) in elevation (Minckley 1973, Hendrickson and Varela-Romero 1989). Historical collections occurred in Baja California and Sonora, Mexico, and in the United States in California and Arizona.

Since the 19th century, desert pupfish habitat has been impacted by streambank erosion, the construction of water impoundments that dewatered downstream habitat, excessive groundwater pumping, the application of pesticides to nearby agricultural areas, and the introduction of non-native aquatic species as both predators and potential competitors (Matsui 1981, Hendrickson and Minckley 1984, Minckley 1985, Schoenherr 1988). The non-native bullfrog may also prove problematic in the management of desert pupfish. The bullfrog is an opportunistic omnivore with a diet that includes fish (Frost 1935, Cohen and Howard 1958, Brooks 1964, McCoy 1967, Clarkson and deVos 1986). Introduced salt cedar (*Tamarisk* spp.) growing adjacent to desert pupfish habitat might cause a lack of water at critical times (Bolster 1990, R. Bransfield, FWS, pers. comm. 1999); however, recent scientific information contradicts the long-held belief that tamarisk consumes more water than native trees (Glenn and Nagler 2005). These threats still occur today and continue to be impacted by increasing human development and demand for water, as well as interactions with predicted trends for warmer, drier, and more extreme hydrological conditions associated with climate change.

Naturally occurring populations of desert pupfish (*Cyprinodon m. macularius* or *C. macularius*) are now restricted in the United States to two streams tributary to, and in shoreline pools and irrigation drains of, the Salton Sea in California (Lau and Boehm 1991). This species is found in Mexico at scattered localities along the Colorado River Delta and in the Laguna Salada basin (Hendrickson and Varela-Romero 1989, Minckley 2000). The Quitobaquito pupfish (*Cyprinodon m. eremus* or *C. eremus*), recently considered to be a separate species, persists in only two populations: one near the United States – Mexico border at Quitobaquito Springs in Organ Pipe Cactus National Monument in Arizona, in the U.S., and the other at Rio Sonoyta in Sonora, Mexico. Collectively, there are 11 extant populations of desert pupfish known in the wild in the United States and Mexico (California = 5, Arizona = 1, and Mexico = 5, Tier 1 populations in the Recovery Plan). Although many re-introductions have been attempted, approximately 16 transplanted populations of the desert pupfish exist in the wild at present, all in Arizona (Tier 2 populations in the Recovery Plan). There is a total of 46 captive or refuge desert pupfish populations (that do not qualify for the Tier 3 category), comprised of 27 in Arizona, 15 in California, and 4 in Sonora, Mexico. The range-wide status of desert pupfish is poor but stable. The fate of the species depends heavily upon future developments in water management of the Salton Sea and Santa de Clara Cienega in Mexico.

### 1.3.1 FR Notice citation announcing initiation of this review:

71 FR 20714, April 21, 2006

### 1.3.2 Listing history

<u>Original Listing</u>
**FR notice:** 51 FR 10842
**Date listed:** March 31, 1986
**Entity listed:** Species, desert pupfish, *Cyprinodon macularius*. Refer to Section 2.3.1.4 for current taxonomy.
**Classification:** Endangered, with critical habitat

### 1.3.3 Associated rulemakings:

Critical habitat was designated in Pima County, Arizona, and Imperial County, California, on March 31, 1986, concurrent with the determination of endangered status, at 51 FR 10842.

### 1.3.4 Review History:

This is the first review for this species since the 1993 recovery plan was published. Therefore, we largely present only information gathered after the recovery plan, or information that was not assessed in the recovery plan. Beyond the original listing process and drafting of the recovery plan, the only significant published Federal reviews of the status of the desert pupfish have occurred during the development of biological opinions as part of section 7 consultation. The most-recent biological opinion containing an evaluation of the status of the species is the USFWS's January 8, 2010, biological opinion on the Proposed Forest Uses and Management of Springs on Gila topminnow and desert pupfish (File no. AESO/SE: 22410-2009-F-0462).

This recent consultation, like many available in our document library at http://www.fws.gov/southwest/es/arizona/Biological.htm, assesses the full status of the species to evaluate the effects of the proposed action, but the narrative describing said status is reduced in scope and tiered to other documents containing more-detailed analyses of status. The document most frequently incorporated by reference is the Desert Pupfish Recovery Plan (USFWS 1993).

The desert pupfish was one of the species addressed in the Imperial Irrigation District Water Transfer biological opinion for the Bureau of Reclamation. The water conservation program considered in this biological opinion was determined to result in reductions in water quality and inflows to the Salton Sea, including irrigation drains that are inhabited by desert pupfish. Imperial Irrigation District is currently working on a desert pupfish refuge pond under the auspices of that opinion (USFWS 2002).

More recently, the USFWS completed a biological opinion for the operation of the U. S. Geological Survey Experimental Ponds that had become occupied by desert pupfish (USFWS 2008). This opinion was recently updated (USFWS 2010a) to address the decommissioning of those ponds, through which over 1,000,000 desert pupfish have been relocated to other extant populations (both native and refuge).

The AGFD has conducted periodic and comprehensive status reviews of the desert pupfish and Gila topminnow in Arizona (Simons 1987, Bagley et al. 1991, Brown and Abarca 1992, Weedman and Young 1997, Voeltz and Bettaso 2003) with funding provided by USFWS via section 6 and State Wildlife Grants.

The California Department of Fish and Game (CDFG) monitors sites in California monthly, except for upper Salt Creek, which is monitored bi-monthly from March through October (Keeney 2006, 2010a). Work on improving the monitoring protocol is being led by CDFG.

A report compiled by the Desert Fishes Team (2003) reviewed the status of *C. m. macularius* in Arizona (also Santa Cruz pupfish, *C. arcuatus*). The information is summarized in tables for historical range, known extirpations, extant populations, reestablishments, recovery and conservation actions, and recommendations. In 2006, the Desert Fishes Team published an analysis of recovery plan implementation in the Gila River basin, which included *C. m. macularius* (Desert Fishes Team 2006).

### 1.3.5 Species' Recovery Priority Number at start of 5-year review: 2C.

A species recovery priority of 2C indicates that the listed entity is a species, the degree of threat is high, the recovery potential is high, and the species is, or may be, in conflict with construction or other development projects or other forms of economic activity.

### 1.3.6 Recovery Plan or Outline

**Name of plan or outline**: *Desert Pupfish Recovery Plan*
**Date issued**: December 8, 1993
**Dates of previous revisions, if applicable**: None

## 2.0 REVIEW ANALYSIS

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

Non-applicable; the desert pupfish is not a designated DPS.

### 2.2 Recovery Criteria

#### 2.2.1 Does the species have a final, approved recovery plan containing objective, measurable criteria?

Yes, the species has a final, approved recovery plan. There are downlisting criteria, but no delisting criteria for the subspecies desert pupfish (*C. m. macularius*). Downlisting or delisting of the Quitobaquito pupfish (*C. m. eremus*) is not expected according to the recovery plan. Because the recovery plan sometimes has different criteria for each subspecies, where there are differences in the discussion below, we will address each subspecies separately. Where there are no differences between the subspecies, we will refer only to desert pupfish. In this review we use the nomenclature under which the species is listed: desert pupfish (*C. m. macularius*) and Quitobaquito pupfish (*C. m. eremus*).

#### 2.2.2 Adequacy of recovery criteria

##### 2.2.2.1 Do the recovery criteria reflect the best available and most up-to date information on the biology of the species and its habitat?

No. Current information shows that the management units in the Salton Sea are different from what is in the recovery plan.

##### 2.2.2.2 Are all of the 5 listing factors that are relevant to the species addressed in the recovery plan?

Yes.

#### 2.2.3 List the recovery criteria as they appear in the recovery plan, and discuss how each criterion has or has not been met, citing information:

The recovery plan treats the two subspecies recognized at that time differently. Insoluble threats and limited habitat are stated as rendering delisting infeasible for either subspecies in the foreseeable future. There are downlisting criteria, but no delisting criteria for the subspecies desert pupfish (*C. m. macularius*). Downlisting or delisting of the single population of Quitobaquito pupfish (*C. m. eremus*), located in southern Arizona on the border, is not expected according to the recovery plan; therefore *Cyprinodon m. eremus* is not discussed further in this section. A Desert Fishes Team report (2006) analyzes and rates recovery plan implementation for *C. m. macularius* in the Gila River basin.

***Recovery criterion 1*** requires that naturally-occurring populations of desert pupfish in the United States and Mexico are secure, including 5 metapopulations at 12 known locations, including:

United States: California

(a) Salton Sink (San Felipe Creek/San Sebastian Marsh, upper Salt Creek, and shoreline pools and irrigation drains of Salton Sea, California);

Mexico: Sonora and Baja California:

(b) Rio Sonoyta, Sonora (*C. m. eremus*);
(c) El Doctor (3 localities) and Santa Clara Slough (2 localities), Sonora;
(d) Laguna Salada, Baja California; and
(e) Cerro Prieto (2 localities), Baja California.

A secure status for the desert pupfish in the United States is defined in the recovery plan as formal protection of habitat and water rights for a minimum of 10 years, with a legally-binding, long-term (greater than 25 years) agreement in place for future management of each naturally-occurring subpopulation, as well as the maintenance of a genetically pure, self-sustaining, stable or increasing (viable) population (USFWS 1993). For these purposes, a viable population is understood to be no fewer than 500 overwintering adults or existing numbers, whichever is greater, in a normal sex ratio with in-situ reproduction and recruitment sufficient to maintain that number (USFWS 1993). For Mexico, formal protection of land and water will be considered to occur when security comparable to that defined for the United States is achieved (USFWS 1993).

Recovery criterion 1 has not been met. Currently, naturally-occurring populations are relatively secure only at San Felipe Creek, California. Table 1 shows the currently known natural populations of desert pupfish. Recovery criterion 1 addresses threat factor A, the present or threatened destruction, modification, or curtailment of the desert pupfish's range, and seeks to minimize the impact of disease and predation (factor C) and other natural or manmade factors (factor E) on the population as a whole.

***Recovery criterion 2*** requires that populations of desert pupfish are reestablished and secure within probable historical range for at least 10 years according to specifications in Task 2 of the Recovery Plan. Task 2 details a 3-tiered plan for protection, reestablishment, and recovery (sufficient to downlist) of desert pupfish (USFWS 1993). Tier 1 is to consist of 7 extant, natural populations representing the original genotypes; Tier 2 is comprised of 28 to 31 replicates of remaining, naturally-occurring stocks re-established in the most natural conditions, within the historical range, and requiring low levels of management; and Tier 3 is made up of 99 to 102 re-established populations in the most natural habitats available after Tier 2 conditions are fulfilled, which may be human-modified to imitate historic conditions and function to optimize balance for genetic diversity and management opportunities (USFWS 1993).

The number of natural and reestablished populations contained in the Task 2 specifications (USFWS 1993: Tables 2 and 3) has not been met in Arizona, California, Baja California, or Sonora (Varela-Romero et al. 2002, Voeltz and Bettaso 2003, Duncan and Tibbits 2008, USFWS files). Most of the reestablished populations are in human-constructed environments (Table 2). The United States refuge populations of Quitobaquito pupfish are all outside of the Rio Sonoyta drainage, and ostensibly outside of historical range. The Desert Fishes Team report (2006) rated the implementation of this task as "low," though multiple reestablishments have occurred since the report (Table 2).

Recovery criterion 2 addresses threat factor A, the present or threatened destruction, modification, or curtailment of the desert pupfish's range, and seeks to minimize the impact of disease and predation (factor C) and other natural or manmade factors (factor E) on the population as a whole.

Table 1. Extant natural populations of desert pupfish (*Cyprinodon macularius*) in the United States and Mexico, by state. *Cyprinodon eremus* populations are in *italics*.

| Arizona | Baja California | California | Sonora |
|---|---|---|---|
| *Quitobaquito* | Cerro Prieto | San Felipe Creek | *Rio Sonoyta* |
| | Laguna Salada | Salt Creek | El Doctor |
| | | Salton Sea | Cienega de Santa Clara |
| | | Hot Mineral Spa Wash | |
| | | Salton Sea irrigation drains | |

Table 2. Reestablished desert pupfish (*Cyprinodon macularius*) populations Arizona.

| | | | | |
|---|---|---|---|---|
| Swamp Springs Canyon[1] | Walnut Spring[1] | Cherry Spring[1] | Tule Creek[1] | Bonita Creek[1] |
| Morgan City Wash[1] | Cold Springs | Secret Spring[1] | Howard Well | Cement Tank Spring[1, 2] |
| Mud Spring – 4 ponds | Lousy Canyon[1] | Muleshoe Hillside Pond[1] | Muleshoe Hot Springs Pond[1] | Bleak Spring[1, 2] |
| Oak Grove Canyon[1] | | | | |

[1] Pupfish population may not be established yet
[2] The Nature Conservancy Safe Harbor Agreement

***Recovery criterion 3*** requires the development and implementation of a protocol to ensure the exchange of genetic material among reestablished populations and maintenance of natural levels of allelic genetic diversity. Recovery criterion 3 is a measure to minimize the adverse effects of gene flow and genetic drift, among other genetic considerations, both in wild and captive-reared individuals. These genetic issues can affect resistance to disease and also overall fitness of a population.

Several reports (Echelle et al. 2007, Koike et al. 2008, Loftis et al. 2009) form the basis for a genetic monitoring plan and largely fulfill this purpose. Loftis et al. (2009) identify five separate management units for *C. m. macularius*, and two for *C. m. eremus*. Further refinements to the recommendations made by Echelle et al. (2007) will lead to a clear genetic management protocol. The Desert Fishes Team (2006) ranked the implementation of this task as “low.” The information in the three reports cited above increases the rating to “high.”

Recovery criterion 3 slightly addresses threat factor A, the present or threatened destruction, modification, or curtailment of the desert pupfish’s range, and primarily addresses factor E, other natural or manmade factors affecting the continued existence of the desert pupfish.

***Recovery criterion 4*** requires that population and genetic monitoring plans, as outlined in the Recovery Plan’s stepdown outline, are devised and implemented to routinely assess the status of all populations. To meet this criterion, twice-annual assessments of population and habitat condition, in conjunction with examination of population genetics at five-year intervals, must be conducted but may be modified once populations have become securely established.

As stated in Section 1.3.4, above, the AGFD has conducted periodic and comprehensive status reviews of the desert pupfish in Arizona (Simons 1987, Bagley et al. 1991, Brown and Abarca 1992, Weedman and Young 1997, Voeltz and Bettaso 2003). The methodology used to assess the status of the desert pupfish in Arizona has been refined by these authors and currently exists as a *de facto* population monitoring protocol in Arizona. Quitobaquito is monitored regularly by Organ Pipe Cactus National Monument staff, following an established protocol (Douglas et al. 2001, Tibbitts 2009). The Rio Sonoyta is sampled annually; no other natural sites in Mexico are regularly surveyed. The CDFG monitors all populations in California monthly or bi-monthly, following an established protocol (Black 1980). These monitoring protocols only partially meet the requirements of recovery criterion 4 and task 5 from the recovery plan. Genetic monitoring and population monitoring and maintenance were ranked as “moderate” implementation by the Desert Fishes Team (2006).

Recovery criterion 4 is not associated with a specific threat factor, although proposing more thorough monitoring plans and management activities relates most closely to Factor D, the inadequacy of existing regulatory mechanisms. This criterion is more properly considered as a method for ensuring that the status of the desert pupfish in the wild and in captivity is accurately assessed, which could address all five listing factors.

## 2.3 Updated Information and Current Species Status

### 2.3.1 Biology and Habitat

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

The desert pupfish has been extensively studied by behaviorists, systematists, physiological ecologists, and geneticists but many aspects of its basic biology remain unstudied. Relatively little new information has been published regarding the biology and life history of the desert pupfish since the approval of the Recovery Plan (USFWS 1993), though Moyle (2002) did summarize currently available information. Work on other species of pupfish has been done, which could be applicable to the conservation of the desert pupfish (Echelle and Dowling 1992, Echelle and Echelle 1998, Echelle et al. 2005). Below is a summary of information published since the 1993 recovery plan:

- Abundance (catch per unit effort (CPUE)) was positively correlated with salinity (Varela-Romero et al. 2002);
- Abundance (CPUE) varies greatly over time (Varela-Romero et al. 2002);
- Abundance (CPUE) was positively correlated with the presence of western mosquitofish (*Gambusia affinis*), cover, pH, and salinity (Martin and Saiki 2005);
- Abundance (CPUE) was negatively correlated with the presence of sailfin molly (*Poecilia latipinna*), porthole livebearer (*Poeciliopsis gracilis*), longjaw mudsucker (*Gillichthys mirabilis*), redbelly tilapia (*Tilapia zillii*), shortfin molly (*Poecilia mexicana*), Mozambique tilapia (*Oreochromis mossambicus*), sediment factor A, and dissolved oxygen (Martin and Saiki 2005);
- Longjaw mudsucker prey on *C. macularius* (Martin and Saiki 2005);
- A single pupfish moved 500m, and 26 others moved lesser distances in various Salton Sea habitats (Sutton 2002);
- Abundance was not correlated with water quality parameters or selenium concentrations (Saiki et al. 2008).

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

The desert pupfish population as a whole is presently stable, though still small, disjunct, and vulnerable to stochastic events that could result in local extirpations. Local populations may be far more variable due to a variety of factors such as amount of habitat, presence of nonnative species, and other threats.

Eleven natural populations of desert pupfish persist; five of these are in Mexico (Table 1). About 16 transplanted populations exist in the wild, all in Arizona (USFWS 1993, Voeltz and Bettaso 2003, USFWS files) (Table 2), though this number fluctuates due to the establishment (and failure) of populations (Moyle 2002).

*Arizona*

No natural populations of *C. m. macularius* remain in Arizona, although numerous captive and wild, reestablished populations currently exist (Table 2 AGFD, unpublished data). These populations have been established on private, municipal, county, state, and Federal lands. Desert pupfish populations stocked on Bureau of Land Management (BLM) and The Nature Conservancy (TNC) lands in the Aravaipa Creek watershed persist in Cement Tank and Bleak springs, but have failed to become established in nearby Parsons Grove Spring (USFWS files, H. Blasius, BLM, pers. comm 2008, AGFD, unpublished data). They have also been established at Oak Grove Canyon on TNC property in the Aravaipa watershed, and have dispersed downstream into waters on BLM lands. Desert pupfish have been established at Mud Springs on the Tonto National Forest, and there are plans to stock them at several additional sites on the Forest in the near future. Desert pupfish have also been successfully established at several wild sites on the Muleshoe Cooperative Management Area. Plans are also in progress for reestablishing desert pupfish to Las Cienegas and San Pedro Riparian National Conservation Areas in southern Arizona. Additional captive sites persist in southern Arizona, with a number of refuge ponds having recently been created under a Safe Harbor Agreement (Table 3).

*California*

Five natural populations persist in California and no reestablished wild populations exist in California or Mexico. There are a total of 15 refuge populations in California (Table 3) (Keeney 2010a). Three of these ponds were stocked with desert pupfish this year, and one more pond is planned to be stocked in fall 2010. A total of six of the ponds have problems with nonnative species, mainly mosquitofish. In addition, desert pupfish are likely extirpated at two more ponds, one of which is being restored (McCallum Pond, Coachella Valley Preserve) (Keeney 2010a).

Desert pupfish numbers in the Salton Sea are relatively low, but they are patchily distributed throughout (Parmenter et al. 2002, Keeney 2010b). While populations in irrigation drains entering the Sea can be abundant (Keeney 2010a), fish populations there are still dominated by nonnative fish (Martin and Saiki 2005). The desert pupfish population in Salt Creek is stable to increasing, and currently has few nonnative species (Keeney 2010a). San Felipe Creek also has a stable to increasing population, and no nonnative fish have been found in recent surveys. Desert pupfish do occur in other areas of the Salton Sink when conditions are suitable, and currently do occur in a wash near Hot Mineral Spa. This population is basically a fifth natural population (Tier 1) of *C. m. macularius* in California.

As part of the research surrounding Salton Sea restoration, a shallow water habitat was constructed near the Alamo Rover (USBR 2005). The project was designed to exclude fish (USBR 2005); however, desert pupfish got into the ponds and flourished (Roberts 2010). The pilot project is over, the site being decommissioned, and the pupfish are being salvaged. Over 1,000,000 desert pupfish were moved to existing and new refuges, and to irrigation drains and other habitats around the Salton Sea (Keeney 2010b).

*Mexico*

In Mexico, five natural populations persist; no reestablished populations persist there. One natural population of *C. m. eremus* persists in Sonora, Mexico, in the Rio Sonoyta. Three refuge populations have been established in the last few years in ponds built in Sonora at Intercultural Center for the Study of Deserts and Oceans, Pinacate, and in Sonoyta (Duncan and Tibbitts 2008).

Additionally, *C. m. eremus* was stocked into the Quitovac Spring and ponds at Ejido Quitovac in 2007. Quitovac is within the Rio Guadelupe drainage, rather than the Rio Sonoyta drainage, and thus is outside of known historical range. The Rio Guadelupe is the next drainage to the east, and very rarely, if ever, flows to the Sea of Cortez. The springs at Quitovac are faunistically similar to the Rio Sonoyta, in that they contain the Rio Sonoyta mud turtle (*Kinosternon sonoriensis sonoytae*), which only occurs in the Rio Sonoyta and Rio Guadelupe drainages (Rosen 2003). The northern divide between the two watersheds is very subtle in the headwaters.

Survey results for Mexico pupfish localities with species presence after each site (species codes are in Appendix A):

1993 – Abarca et al. 1993

- MODE terminus backwater – CYMAMA
- El Campo – CYMAMA and other species
- Hunters Camp – CYMAMA

1994 – Zengel and Glenn 1996

- MODE terminus – CYCA, GAAF, *Lepomis*
- El Campo – MISA, CYLU, GAAF, *Lepomis*
- Hunters Camp – CYMAMA abundant, MISA, CYLU, GAAF, *Lepomis*
- Lagunas Truck, Rafael, Pelicano – MISA, GAAF, CYLU, MUCE

1996 – Campoy-Favela 1996

- Hunters Camp – 1 CYMAMA with POLA, GAAF
- El Doctor – 1 CYMAMA with POLA, GAAF
- Rancho La Atlantida – 1 CYMAMA with POLA, GAAF

1996-97 – Varela-Romero et al. 2002

- El Doctor – CYMAMA, GAAF, POLA, TIZI present
- Flor del Desierto – CYMAMA, GAAF, POLA, GIMI, TIZI, LECY present

- Hunters Camp – No CYMAMA; GAAF, POLA, MISA, LECY Present
- Cerro Prieto – CYMAMA present
- Cerro Prieto near Nayarit – CYLU, GAAF, POLA, TIZI, MISA present
- MODE – CYMAMA, POLA, GAAF, TIZI present

1997 – Minckley, Bagley, Knowles (Bagley et al. 1997)

- Rio Sonoyta – CYMAER, AGCH, GAAF all common
- El Doctor – CYMAMA, GAAF, POLA present
- Flor del Desierto – CYMAMA, GAAF, POLA present
- Hunters Camp – No CYMAMA; GAAF, POLA, CYCA, CYLU, *Lepomis* Present
- Santa Clara Slough – CYMAMA, GAAF, POLA present
- Cerro Prieto – CYMAMA, GAAF, POLA, *Tilapia* present
- Cerro Prieto near Nayarit – CYLU, GAAF, POLA, present
- Sierra Las Pintas, north end – dry
- Laguna Salada – CYMAMA

1998 – USFWS AESO EC (Velasco 1998)

- Flor del Desierto – CYMA, POLA, GAAF
- Rio Hardy-Campo Mosqueda – GAAF, POLA
- Rio Hardy-El Mayor – GAAF, POLA, *Lepomis*
- Rio Colorado @ Highway 2 – GAAF, CYLU, *Lepomis*, MUCE
- MODE – CYLU, POLA, GAAF, *Tilapia*, *Lepomis*
- El Doctor – GAAF, POLA, *Tilapia*
- Santa Clara Slough – GAAF, POLA

2000 – Minckley 2000

- El Doctor – CYMAMA, GAAF, POLA, *Tilapia*
- Flor del Desierto – CYMAMA, GAAF, POLA, *Tilapia*, ICNA, crayfish, LECY
- La Pila – 17 CYMAMA
- MODE – CYLU
- Cerro Prieto – CYMAMA

2007 – Minckley, Timmons, Duncan (Duncan 2007)

- Laguna Salada – juvenile and adult CYMAMA common in springheads

2007-2010 – Izaguirre-Pompa, Minckley, Timmons, Rosen, Duncan, Caldwell (Service files)

- Rio Sonoyta – CYMAER numerous, AGCH uncommon then disappeared in 2009, AMNA uncommon then disappeared in 2008, GAAF very common then absent in 2010

Table 3. Known extant refuge or captive populations of desert pupfish (*Cyprinodon macularius*) in the U.S. and Mexico. *Cyprinodon m. eremus* sites are in *italics*.

| Arizona | Baja California | California[2] | Sonora |
|---|---|---|---|
| Lulu Walker Elementary School | | Anza Borrego State Park – 3 ponds | *Pinacate Reserva* |
| International Wildlife Museum | | Oasis Springs Ecological Reserve – 2 ponds | *Intercultural Center for the Study of Deserts and Oceans* |
| Dexter National Fish Hatchery[1] | | Salton Sea State Recreation Area | *Colegio de Bachilleres* |
| McDowell Mountain Regional Park[3] | | Dos Palmas Reserve – 2 ponds | *Quitovac*[1] |
| Robbins Butte Wildlife Management Area – 2 ponds[3] | | Living Desert Museum – 4 ponds | |
| TNC Lower San Pedro Preserve[3] | | University-California Riverside | |
| Audubon Society Appleton-Whittell Research Ranch[3] | | Borrego Springs High School – 2 ponds | |
| *Organ Pipe Cactus National Monument* | | | |
| Cibola NWR | | | |
| *Hernbrode*[1, 3] | | | |
| Arizona-Sonora Desert Museum | | | |
| *ASDM* | | | |
| Desert Botanical Garden | | | |
| *La Cienega at Organ Pipe*[1] | | | |
| Apache Elementary School | | | |
| Arizona Historical Society | | | |
| Phoenix Zoo – 2 ponds | | | |
| Spur Cross Solar Oasis[3] | | | |
| Libby Elementary School | | | |
| Bill Williams NWR | | | |
| *Cordery Pond*[1] | | | |
| Deer Valley High School | | | |
| *Cabeza Prieta NWR*[1] | | | |
| *Onofryton Pond*[1, 3] | | | |
| Imperial NWR | | | |
| Scottsdale Community College | | | |
| Boyce-Thompson Arboretum | | | |

[1] Extra-limital
[2] Keeney 2010a
[3] AGFD Safe Harbor

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

Much of the research on desert pupfish since the 1993 recovery plan addresses genetics issues and the taxonomy of the *C. macularius* group. Since the isolated nature of desert pupfish populations reduces the flow of genes between sites; inbreeding and genetic drift can be reasonably expected to occur without intervention (Turner 1983, Echelle et al. 2007, Koike et al 2008, Loftis et al. 2009). Turner (1983) compared samples from desert pupfish populations at six localities and detected allozyme differences among stocks from Salton Sea, Cienega de Santa Clara, and Quitobaquito Spring (the latter now recognized as a distinct species [below]). The overall level of differentiation was low and in the range of within-population comparisons in other teleosts. The recovery plan suggested expanding Turner's (1983) data to include populations from Rio Sonoyta, additional localities on the lower Colorado River Delta, and individual populations in California and include analysis of mitochondrial DNA. Resultant information could be used to determine levels of differentiation among all known natural populations of desert pupfish and guide development of a protocol for exchange of genetic material among reestablished populations (USFWS 1993).

Similar to Turner (1983), Dunham and Minckley (1998) also looked at allozyme variation, but in both wild and captive populations of *C. macularius*. In their paper, they documented that some of the captive populations were almost all founded with less than 300 fish from the wild, or from another captive site. The Living Desert captive population was founded with 10 pupfish from the Salton Sea (Dunham and Minckley 1998).

Dunham and Minckley (1998) found clear variation among the various captive and wild *C. m. macularius* populations that they tested. They found differences between the Salton Sea and Colorado River delta populations, and between the refuge populations that were founded from those two populations. Heterozygosity among the captive populations was strongly associated with the size of the founder population, but not with time since founding, habitat size, or number of founding events (Dunham and Minckley 1998). They also found significant genetic differences between wild and captive populations.

Results from subsequent studies (Echelle et al. 2000, Echelle et al. 2007, Loftis 2007, Koike et al. 2008, Loftis et al. 2009) have helped to quantify the genetics of both *C. m. macularius* and *C. m. eremus* wild and refuge populations. Echelle et al. (2000) looked at the variation in mitochondrial DNA in 11 natural *C. macularius* populations and 1 captive population. They found that the populations in the Rio Sonoyta and Quitobaquito did not share any haplotypes with the Salton Sea or Colorado River delta samples. There were small mitochondrial DNA differences between the Salton Sea and Colorado River delta, but not between Rio Sonoyta and Quitobaquito. Similar to Dunham and

Minckley (1998), they also found the captive population at Dexter had significantly diverged from its wild, parent stock.

Based on their work on the natural populations, Loftis (2007) and Echelle et al. (2007) recommended several management units. For *C. m. eremus* they recommended that the Rio Sonoyta and Quitobaquito populations be managed separately (Echelle at al. 2000). They recommended five management units for *C. m. macularius*: Laguna Salada, Cerro Prieto, Cienega de Santa Clara/El Doctor, San Felipe Creek, and the rest of the Salton Sea system (Echelle et al. 2007, Loftis et al. 2009).

Dunham and Minckley (1998), Echelle et al. (2007), and Koike et al. (2008) found that captive populations of desert pupfish had diverged significantly from wild fish. The global refuge populations for both *C. m. macularius* and *C. m. eremus* represent the genetics of the wild populations relatively well (Koike et al. 2008). Refuges of *C. m. eremus* best reflected the wild populations, largely due to the recent founding of one refuge and another refuge that had two stockings of wild fish. However, the genetic diversity for most individual lineages and their refuges for *C. m. macularius* and *C. m. eremus* is well below that of wild populations, and these lineages have markedly low levels of microsatellite diversity (Koike et al. 2008). Low genetic diversity, especially regarding rare alleles, can affect the expression of loci that determine quantitative traits, which are the targets of natural selection (Lande 1980, Koike et al. 2008). Refuge (or lineage) age, number of founding events, number of founders, and supplementation all affect genetic diversity of refuge populations and must be considered for long-term management (Echelle et al. 2007, Koike et al. 2008).

**2.3.1.4 Taxonomic classification or changes in nomenclature**:

The name desert pupfish is often incorrectly applied to all 10 pupfish species in the American Southwest (Williams et al. 1989, Pister 1996). In the geographic area addressed by this review, there are now three pupfish species (see below). The revised taxonomic relationships between these species are not reflected in the final rule (51 FR 10842), the Recovery Plan (FWS 1993), or the list of listed species in the Code of Federal Regulations (CFR) (50 CFR 17.11). Currently, the USFWS is in the process of correcting the list of listed species in the CFR to update the desert pupfish's taxonomic status.

The desert pupfish complex was historically comprised of two subspecies, the nominal desert pupfish (*C. m. macularius*), and the Quitobaquito pupfish (*C. m. eremus*), and an undescribed species, the Monkey Spring pupfish (*Cyprinodon* sp.) (USFWS 1993). The subspecies are now recognized as three separate species: the desert pupfish (*C. macularius*), the Sonoyta (Quitobaquito) pupfish (*C. eremus*) (Echelle et al. 2000), and the undescribed Monkey Springs pupfish which has since been described and renamed the Santa Cruz pupfish (*C. arcuatus*)

(Minckley et al. 2002, Fishbase.org 2010a, b, c; Scharpf 2010). These are part of the western clade of pupfishes (Echelle and Echelle 1993, Echelle 1998).

Echelle (1998) and Echelle et al. (2000) studied mitochondrial DNA (mtDNA) variation among populations of pupfish traditionally classified as *C. macularius* to provide a basis for recommendations regarding conservation genetics of the species. The mtDNA haplotypes of the Rio Sonoyta Basin/Quitobaquito Springs populations and those of the Salton Sea/Lower Colorado River Basin populations suggest long, mutually exclusive evolutionary histories (Niegel and Avise 1986) for these two groups. The differences between the two species in their mtDNA haplotype frequency are highly significant (Echelle et al. 2000). Geological history and the mtDNA genealogy show that this complex comprises separate entities that have been diverging for about 100,000 years (Ives 1964, Echelle et al. 2000). They are diagnosable on the basis of two independent characters, mtDNA and male breeding color. The two groups clearly qualify as species under the evolutionary species concept (Wiley 1978, Frost and Hillis 1990) and the various forms (Mayden and Wood 1995) of the phylogenetic species concept. This apparently recent speciation explains the lack of allelic differences found by Turner (1983) and low overall mtDNA divergence found by Dunham and Minckley (1998). These findings are similar to other *Cyprinodon* groups (Echelle and Dowling 1992, Echelle et al. 2000). Therefore Echelle et al. (2000) concluded that there are two species of desert pupfish, the Sonoyta pupfish (= Quitobaquito pupfish; *C. eremus*) in the Rio Sonoyta basin and the desert pupfish (*C. macularius*) in the lower Colorado River basin, the Salton Sink, and the Laguna Salada.

More recent work (Echelle et al. 2007, Koike et al. 2008) provided further evidence that *C. macularius* and *C. eremus* are separate species. Results from microsatellites assays attribute 23 percent of microsatellite diversity to differences between the two species (Echelle et al. 2007). There was a small, but statistically significant part of the microsatellite diversity attributed to variation among the Salton Sea populations and the Colorado River delta populations. For *C. eremus*, there were differences in microsatellites between the two populations, but they were not significant (Echelle et al. 2007). They found no genetic evidence of separate evolutionarily significant units for either species. However, they recommended the recognition of two management units for *C. eremus* (Quitobaquito and Rio Sonoyta) and five for *C. macularius*, three in the Colorado River delta (Laguna Salada, Cerro Prieto, and Cienega de Santa Clara/El Doctor) and two in the Salton Sea (San Felipe Creek/San Sebastian Marsh and Salton Sea). They state that the loss of any one of the management units would be a significant step toward extinction of the species (Echelle et al. 2007).

The ranges of two subspecies of mud turtle may reflect a shared history of habitat availability with the desert pupfish. Based on distinct morphometry (Iverson 1981) and mtDNA (Rosen 2003), the Sonoyta mud turtle (*Kinosternon sonoriense longifemorale*) is separated from the nominate subspecies (Sonora mud turtle = *K.*

*s. sonoriense*). The range of the Sonoyta mud turtle completely overlaps that of *C. eremus*. The historical range of the Sonora mud turtle overlaps the historical range of *C. arcuatus* and *C. macularius* in southern Arizona. The similar biogeographic pattern of the two Rio Sonoyta basin species further supports the specific status of *C. eremus*.

Minckley et al. (2002) split off a portion of what was once considered *C. macularius*. The Monkey Spring pupfish was long considered a separate, but undescribed species (*Cyprinodon* sp.) (Minckley1973). Minckley et al. (2002) named this undescribed species the Santa Cruz pupfish (*C. arcuatus*). The common name indicates that they included specimens from the mainstem Santa Cruz River in the Tucson basin that had been originally referred to as *C. macularius* (Minckley 1973, Minckley et al. 2002). *Cyprinodon arcuatus* differs from *C. macularius* and *C. eremus* by the following: "distinctive, dorsal body surface, which is highly convex before the dorsal fin but changing abruptly at the dorsal origin into a deep, postdorsal concavity most developed in breeding males; absence in nuptial males of distinctive yellow or orange pigment on either caudal fin or peduncle; weak development of lepidodonts, and modally six preopercular pores (Minckley et al 2002:700)."

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

The currently recognized historical range of *C. macularius* has changed due to the taxonomic changes of the last 10 years. The recognition and naming of *C. eremus* and *C. arcuatus* as separate species removed the Rio Sonoyta and Santa Cruz River basins from the previously known historical range of the desert pupfish. These two basins are relatively small when compared to the remaining historical range of the desert pupfish in the Gila River basin and lower Colorado River basin.

The spatial distribution of the desert pupfish remains relatively stable, though the present historical range represents only a small, peripheral, and fragmented portion of the species' former distribution within the lower Colorado, Rio Sonoyta, and Gila River systems (Table 4). New populations, both wild and refuge (captive) populations, continue to be established in Arizona (Robinson 2009). The populations in and around the Salton Sea irrigation drains and shoreline pools wax and wane over time (Martin and Saiki 2005).

| Table 4. Spatial distribution of wild desert pupfish (*Cyprinodon macularius*) complex populations. | | |
|---|---|---|
| Basin | Historical[1] | Known[1] |
| Gila River basin | Salt, San Pedro, Santa Cruz, Gila Rivers | extirpated |
| Lower Colorado River | River below Needles | El Doctor, Cerro Prieto, Cienega de Santa Clara |
| Rio Sonoyta/ Puerto Penasco | present | Rio Sonoyta |
| Laguna Salada | present | present |
| Quitobaquito | present | present |
| Salton Sea | present | San Felipe/San Sebastian, Salt Creek, Salton Sea, irrigation drains, Hot Mineral Spa Wash |
| [1] From 1993 recovery plan, natural populations | | |

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

A great deal of nominal desert pupfish habitat exists, though the increasing incidence of nonnative competitive and predatory fishes and ranid frogs have rendered many sites unsuitable for reestablishment, absent appreciable renovations through removal of nonnative species (Voeltz and Bettaso 2003). We discussed earlier the water level problems that occurred at Quitobaquito and water quality issues at the Salton Sea are evaluated under the Five Factor Analysis. The status of the Cienega de Santa Clara and its main water source, the Main Outlet Drain Extension (MODE) canal, is still uncertain, pending negotiations on the status of MODE water and the desalinization plant (Yuma Desalting Plant/Cienega de Santa Clara Working Group 2005, Hucklebridge et al. 2010). Based on the outcome of negotiations, final conditions could include any of the following: less water may go to the Cienega, water may come from elsewhere, and the water may be more saline than it is currently.

**2.3.1.7 Conservation Measures**:

<u>Quitobaquito pupfish (*C. m. eremus)*</u>

Beginning in 2005, and through 2006 to 2009, the surface elevation of Quitobaquito Pond fell to extremely low levels, unprecedented in the known history of the pond since it was dredged and deepened in 1962. Normally averaging about 63cm to 98cm deep and about 2,500 $m^2$ in surface area, by 2008 the pond averaged 4cm" deep and 40% of its normal surface area. This loss of surface area and total water volume presented imminent threats to the Quitobaquito pupfish and the Sonoyta mud turtle. Numerous actions taken by Organ Pipe Cactus National Monument during 2007-2009 to ameliorate the threats to Quitobaquito Pond include:

- Evacuated desert pupfish and Sonoyta mud turtles in 2007 and 2008;
- Rehabilitated the Northeast Spring collection system in 2007;
- Trucked over 314,000 liters of water to the pond in midsummer 2008;
- Rehabilitated pond berm;
- Removed desert pupfish and turtles, dried out the pond, and then relined the bottom and berm at the southeast corner of the pond.

It appears that these actions, and especially the last one, have stopped the leak. The lowest pond level, and during the last action, was minus 66cm". The pond level has steadily increased to minus 41cm, during a period of warmer than normal temperatures and no rainfall. The efforts of Organ Pipe Cactus National Monument and partners appear to have succeeded. Summaries of actions taken can be found in several different documents (Tibbitts 2008, 2009, USNPS 2009).

Even with the water level problems at Quitobaquito pond, pupfish capture numbers have stayed within historical limits for the September survey (Tibbitts 2009). Desert pupfish captured in 2005 were the second-lowest number recorded (1,358); however, pond levels did not become particularly problematic until 2007. The numbers of pupfish captured in 2006 was average (2,982), but in 2007, when the pond level began dropping precipitously, the greatest number ever was captured (5,361). The lower water probably provided better desert pupfish habitat (Tibbitts 2008). In 2008 however, low water level allowed sampling only the moat and channel (1,692 captures). No survey was done in 2009.

Desert pupfish (*C. m. macularius*)

The completion of Safe harbor Agreements that include the desert pupfish with the Arizona Department of Transportation, The Nature Conservancy, and the AGFD has provided opportunities to expand desert pupfish populations on non-Federal lands (Table 3). Additional reestablishment and recovery projects have occurred and others are being planned (Robinson 2009).

Our information indicates that, more than 63 formal consultations have been completed or are underway for actions affecting desert pupfish in Arizona. The majority of these opinions concerned the effects of livestock grazing, roads and bridges, agency planning, or recovery. The remaining 47 percent of consultations dealt with timber harvest, fire, flooding, recreation, realty, water development, and water quality issues. Conservation measures that have been implemented in these biological opinions, largely on actions that reestablished the species include: fencing from livestock and vehicles, maintaining of aquatic vegetation, dredging, fish salvaging, creating signage, establishing buffer zones, coordinating fire suppression activities, and monitoring water levels.

A total of 17 formal or informal consultations have been conducted relative to the desert pupfish in California. These consultations covered an array of activities from pest control programs and irrigation drain maintenance to a habitat conservation plan and a major water transfer. USFWS and CDFG staff continue to coordinate on these activities and on the oversight of the various refuge ponds to ensure appropriate consideration of the pupfish occurs in projects that may affect it.

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

#### 2.3.2.1 Present or threatened destruction, modification or curtailment of its habitat or range (Factor A):

Many natural and reestablished desert pupfish populations are imperiled by one or more threats. Threats to the species relating to destruction or curtailment of habitat include loss and degradation of suitable habitat through ground water pumping or water diversion; contamination from agricultural return flows, as well as other contaminants, and physical changes to water properties involving suitable water quality (71 FR 20714, USFWS 1986, Moyle 2002, Martin and Saiki 2005, Echelle et al. 2007, Minckley and Marsh 2009). On Federal lands, consultations have addressed effects of grazing, roads and bridges, agency planning, fire, flooding, recreation, pest control programs, irrigation drain maintenance, water transfers and water development as potential threats to desert pupfish habitat. Although effects from these threats continue to be moderated for the desert pupfish, biologically, impacts from these threats individually and collectively can create fragmented populations of poorer quality habitat that are small and restricted in range, which can further endanger the desert pupfish.

<u>Water</u>

*Water loss*

Groundwater extraction was considered a threat in the listing (51 FR 10842) and in the recovery plan (USFWS 1993). It is still considered a threat; especially at Quitobaquito, Rio Sonoyta (Brown 1991), and El Doctor (P. Reinthal, University of Arizona, pers. comm.). Water extraction removes and degrades habitat, leaving higher concentrations of salts, toxic contaminants, and sediment in the remaining volumes of water and lower amounts of dissolved oxygen, and thus interacts with other compounding threats. Water reductions could lead to less shallow-water habitat preferred by the desert pupfish. Slight increases in salinity could benefit desert pupfish, by reducing populations of problematic nonnative fishes. However, if salinity keeps increasing, wetland areas may become unsuitable even for pupfish. Any change to the water budget at Cienega de Santa Clara could be detrimental to the desert pupfish there. Groundwater withdrawal in the Rio Sonoyta drainage has exceeded recharge for decades. In addition, the pumping capacity is about twice of what is withdrawn in an average year (Brown 1991, Pearson and Conner 2000).

*Watershed Health*
Watershed condition has been and continues to be a concern over most of the Southwest. Recreational pursuits that have the potential to increase soil erosion (i.e. off-highway vehicles (OHVs)) are a concern for desert pupfish because of their impacts to watershed health, rather than any direct effects. Overgrazing and historically extensive logging combined with climatic events (drought followed by rain events), have led to increased erosion and deeper channelization (Miller 1961, Bahre 1991), which do not provide the more shallow, clear, and vegetatively complex wetlands preferred by the desert pupfish (Hanes 1996). Extensive logging is no longer a threat to desert pupfish or their habitats. Improper grazing at a watershed level probably does not impact desert pupfish populations anymore, except at the Rio Sonoyta. Grazing of occupied sites still occurs in Mexico and the United States. However, grazing in the United States is better managed and much less of a concern for its impacts to desert pupfish habitat. Urbanization and other human activities can and continue to impact watershed health and functioning.

*Contaminants*
Environmental contaminants, such as heavy metals, accumulating in water sources were given as threats at the time of listing, particularly in the form of mercury. At this time, selenium seems to be the element of most concern for fishes in the Salton Sea (Saiki 1990, California Regional Water Quality Control Board 1991, McClurg 1994, Saiki et al. 2008). In addition to conditions of elevated salinity, contaminants are still present in irrigation drains entering the Salton Sea. These include problematic levels of heavy metals and organochlorines entering the Salton Sea, and effects to dissolved oxygen in the Salton Sea (Saiki 1990, Matsui et al. 1992). Salinity in the Salton Sea is expected to continue increasing (Saiki 1990, Matsui et al. 1992) to the point the Sea will be inhospitable for all fish (California Regional Water Quality Control Board 1991, McClurg 1994), unless planned restoration actions occur.

Grazing

Livestock grazing was not mentioned as a threat in the final rule (51 FR 10842), though habitat modifications from grazing was mentioned in the recovery plan (USFWS 1993). The small size and high physical tolerance of the desert pupfish allow it to exist in small amounts of water spanning a wide variety of extreme habitat and water quality conditions (USFWS 1993). Due to the scarcity of water in the desert pupfish's desert habitat and the tendency for cattle to congregate in watered areas, cattle are attracted to desert pupfish habitats that can lead to local impacts quickly. Low water conditions combined with congregations of cattle activity (grazing, watering, hoof action) can lead to additional reductions in water, physiological effects of reduced water quality, bank trampling, fragmentation of contiguous water, isolation/stranding and trampling of fish and eggs (Roberts and White 1992), and loss of habitat through de-watering. Long-term or seasonal drought can also exacerbate these conditions. Round-up of trespass cattle within these small enclosed areas could cause cattle congregations to increase their hoof action and cause movement into fish habitat. Cattle can cause disturbance, a decline in water quality, and mortality of fish and desert pupfish eggs, particularly at the perimeter of ponds, springs, wells, and shallow wetland areas, by reducing the distribution and abundance of water and isolating fish and eggs into inhospitable areas (Kauffman and Krueger 1984, Fleischner 1994, and Belsky et al. 1999). Carefully controlled grazing around some of the small pond habitats as a tool to manage problematic aquatic vegetation could actually be beneficial to the desert pupfish (Kodric-Brown and Brown 2008). Although impacts from livestock grazing have been problematic in some areas, as a result of consultations many of the impacts have been alleviated through fencing and grazing rotations.

**2.3.2.2 Overutilization for commercial, recreational, scientific, or educational purposes (Factor B):**

There is no new information to suggest that overutilization for commercial, recreational, scientific, or educational purposes are threats. Threats relating to this factor were not identified in the Final Rule (51 FR 10842) or recovery plan (USFWS 1993).

**2.3.2.3 Disease or predation (Factor C):**

Desert pupfish are susceptible to parasites and predation and competition from nonnative fish and other species. Desert pupfish are known to suffer infestations of anchor worm (*Lernea* spp.) (51 FR 10842) (Robinson 2009). Miller and Fuiman (1987) noted a nematode parasite present in desert pupfish collected from Quitobaquito Springs in Organ Pipe Cactus National Monument and hypothesized, after Cox (1966) that the parasites resembled a nematode known from birds and that waterfowl or shorebirds were a possible vector for introduction to the desert pupfish. It is therefore conceivable that many desert pupfish populations are at risk of infestation by this parasite. However, the specific effects to individual desert pupfish or populations are unknown. *Lernea* can kill its host, though largely through secondary infections.

Predation and competition from nonnative fish have been identified as main causes of the decline of the species (51 FR 10842, USFWS 1993). Nonnative fish are still a major threat to the desert pupfish at this time. Martin and Saiki (2009) found the remains of *C. m. macularius* in the gastrointestinal contents of one longjaw mudsucker. In addition they found unidentifiable fish remains in the gastrointestinal contents of sailfin molly, porthole livebearer, longjaw mudsucker, redbelly tilapia, Mozambique tilapia, and western mosquitofish. In an earlier study (2005) they found the abundance of *C. m. macularius* to be inversely related to the abundance of nonnative fish.

It has long been assumed that western mosquitofish have a negative impact on desert pupfish (Deacon and Minckley 1974, USFWS 1993), through similar mechanisms by which they affect other small fishes, such as competition for food and the predacious habits of mosquito fish upon young fish, as well as fin damage under crowded conditions (Meffe at al. 1983, Meffe 1985). Martin and Saiki (2009) found unidentifiable fish remains in western mosquitofish. They also believed there was significant dietary overlap between desert pupfish and western mosquitofish. To the contrary however, Martin and Saiki (2005) also found the abundance of desert pupfish was positively correlated with the presence of western mosquitofish. We surmise that this result stems from the high tolerance of both species to poor water quality and from competition with the many other nonnative fish individuals present in shared habitats. Because nonnative aquatic species are present in many occupied or potential desert pupfish habitats and nonnative aquatic species are exceedingly difficult to get rid of once established, nonnative aquatic species continue to be a major threat to the conservation of the desert pupfish.

**2.3.2.4 Inadequacy of existing regulatory mechanisms (Factor D):**

Regulatory mechanisms exist in much the same state as at the time of listing (51 FR 10842), though the application of recent case law may result in reduced consideration of impacts to isolated waters containing desert pupfish. Desert pupfish are listed as endangered in Mexico (Peligro de extincion), in California under the California Endangered Species Act (CESA), and a species of greatest conservation need in Arizona (no legal protection). The CESA prohibits the taking of state listed species, except as allowed by state law. Desert pupfish are a protected species in Arizona. However, the state has no authority to protect pupfish habitat.

Even though the desert pupfish is listed as endangered in Mexico, several desert pupfish sites are within Biosphere Reserves, and there was a groundwater pumping moratorium in the Sonoyta Valley (Pearson and Conner 2000), enforcement of environmental laws in Mexico is limited at best. Enforcement in Mexico is increasing, but still lags behind the United States (Diaz 2000, Behr 2003, Bailey 2004, Ruanova and Feliz-Saul 2007).

Many desert pupfish localities are on public lands and as such, are protected to some extent from adverse effects such as draining or removal since section 7 requirements Endangered Species Act (ESA) would apply. Sites on non-Federal land without a Federal nexus would be subject to ESA sections 9 and 10 for any actions that could lead to take of desert pupfish.

The main Federal regulatory program protecting wetlands in the United States is section 404 of the Clean Water Act (CWA), implemented by the U.S. Army Corps of Engineers (Corps). Under section 404, any party wishing to discharge fill material into "waters of the United States" must obtain a permit from the Corps.

On January 9, 2001, the U.S. Supreme Court issued a decision in the case (Solid Waste Agency of Northern Cook County [SWANCC] v. United States Army Corps of Engineers [531 U.S. 159, 2001]). The Court determined that the Corps' authority under the CWA did not extend to isolated wetlands if they are not "adjacent" to navigable waters. Many of the remaining sites containing or suitable for the reestablishment of desert pupfish are isolated and nonadjacent to navigable waters and thus, may no longer be subject to the discretionary authority of the Corps. This finding potentially removes protection of some desert pupfish habitats.

Other Federal laws that may influence desert pupfish and their habitat include: National Environmental Policy Act, Federal Clean Water Act, Sikes Act, National Park Service Organic Act, National Forest Service Management Act, Federal Land Policy and Management Act and the Lacey Act. Other than the ESA, only CESA provides any significant protection to desert pupfish and their habitat. Therefore, the desert pupfish is not adequately protected by any other existing regulatory mechanisms.

#### 2.3.2.5 Other natural or manmade factors affecting its continued existence (Factor E):

The only threat discussed in the final rule (51 FR 10842) was the exotic weed *Hydrilla* and how control actions would likely be detrimental to pupfish habitat. The only new threat identified is that endocrine disruptors have been noted in the Salton Sea irrigation drains (C. Roberts, USFWS, pers. comm., 4 August 2010).

Many occupied pupfish localities are small, fragmented, and highly threatened. The theory of island biogeography can be applied to these isolated habitat remnants, as they function similarly (Meffe 1983, Laurenson and Hocutt 1985). Species on islands are more prone to extinctions than continental areas that are similar in size (MacArthur and Wilson 1967) because smaller areas tend to have fewer resources and fewer opportunities for exchange of genetic material from other desert pupfish populations than larger areas of habitat. As the genetic pool becomes more separated and limited, a population trapped in a small pond has decreasing chances of developing genetic diversity and potential adaption to changes, and of sustaining environmental stochasticity in the long run. Based on the isolated nature of desert pupfish populations, when only a few populations of a rare fish species occur, the extirpation of one of those populations can be almost as critical as that of a recognized species extinction (Meffe 1983).

### 2.3.3 Climate Change

That much of the American southwest has experienced serious drought recently is well known. What is known with far less certainty is how long droughts may last. State-of-the-art climate science does not yet support multi-year or decade-scale drought predictions. However, instrumental and paleoclimate records from the Southwest indicate that the region has a history of multi-year and multi-decade drought (Hereford et al. 2002, Sheppard et al. 2002, Jacobs et al. 2005). Multi-decade drought in the Southwest is controlled primarily by persistent Pacific Ocean-atmosphere interactions, which have a strong effect on winter precipitation (Brown and Comrie 2004, Schneider and Cornuelle 2005). Also, persistent Atlantic Ocean circulation is theorized to have a role in multi-decadal drought in the Southwest, particularly with respect to summer precipitation (Gray et al. 2003, McCabe et al. 2004). Given these multi-decade "regimes" of ocean circulation, and the severity and persistence of the present multi-year drought, there is a fair likelihood that the current drought will persist for many more years (Stine 1994, Seager et al. 2007), albeit with periods of high year-to-year precipitation variability characteristic of Southwest climate.

The information on how climate change might impact southeastern Arizona is less certain than current drought predictions. However, virtually all climate change scenarios predict that the American southwest will get warmer during the 21st century (IPCC 2001, 2007). Precipitation predictions show a greater range of possibilities, depending on the model and emissions scenario, though precipitation is likely to be less (USGCRP 2001, Seager et al. 2007). To maintain the present water balance with warmer temperatures and all other biotic and abiotic factors constant, precipitation will need to increase to keep pace with the increased evaporation and transpiration caused by warmer temperatures.

Key projections to keep in mind include:

• decreased snowpack — an increasing fraction of winter precipitation could fall as rain instead of snow, periods of snowpack accumulation could be shorter, and snowpacks could be smaller; ironically, due to changes in snow-precipitation characteristics, runoff may decrease even if total precipitation increases (Garfin 2005, Seager et al. 2007);

• earlier snowmelt — increased minimum winter and spring temperatures could melt snowpack's sooner, causing peak water flows to occur much sooner than the historical spring and summer peak flows (Stewart et al. 2004);

• enhanced hydrologic cycle—in a warmer world an enhanced hydrologic cycle is expected; flood extremes could be more common causing more large floods; droughts may be more intense, frequent, and longer-lasting (Seager et al. 2007).

Continuing drought and climate change, when added to the historical and continuing threats, will make native aquatic species conservation in the American southwest even more difficult (Duncan and Garfin 2006). The impact of site desiccation to fish is obvious. Frogs may be able to move to another site. Many less obvious effects could occur with drought and a warmer climate. A site with reduced streamflow, or a pond or pool with low water levels could become fishless due to reduced dissolved oxygen. Nonindigenous aquatic species may become more restricted in distribution as well; however, both native and nonindigenous species will be competing for remaining aquatic habitats, and extensive case history suggests that nonindigenous species will win.

Average Northern Hemisphere temperatures during the second half of the 20th century were very likely higher than during any other 50-year period in the last 500 years and likely the highest in at least the past 1300 years (IPCC 2007). It is very likely that over the past 50 years cold days, cold nights and frosts have become less frequent over most land areas, and hot days and hot nights have become more frequent (IPCC 2007). Data suggest that heat waves are occurring more often over most land areas, and the frequency of heavy precipitation events has increased over most areas (IPCC 2007).

The International Panel on Climate Change (IPCC) (2007) predicts that changes in the global climate system during the 21st century are very likely to be larger than those observed during the 20th century. For the next two decades a warming of about 0.2°C (0.4°F) per decade is projected (IPCC 2007). Afterwards, temperature projections increasingly depend on specific emission scenarios (IPCC 2007). Various emissions scenarios suggest that by the end of the $21^{st}$ century, average global temperatures are expected to increase 0.6°C to 4.0°C (1.1°F to 7.2°F) with the greatest warming expected over land (IPCC 2007).

Localized projections suggest the southwest may experience the greatest temperature increase of any area in the lower 48 States (IPCC 2007), with warming in southwestern states greatest in the summer (IPCC 2007). The IPCC also predicts hot extremes, heat waves, and heavy precipitation will increase in frequency (IPCC 2007). There is also high confidence that many semi-arid areas like the western United States will suffer a decrease in water resources due to climate change (IPCC 2007), as a result of less annual mean precipitation and reduced length of snow season and snow depth (IPCC 2007). Milly et al. (2005) project a 10–30 percent decrease in precipitation in mid-latitude western North America by the year 2050 based on an ensemble of 12 climate models.

Drought, climate change, and temperature increases will also impact watersheds and subsequently the water bodies in those watersheds. Drought, and especially long-term climate change, will affect how ecosystems and watersheds function. These changes will cause a cascade of ecosystem changes, which may be hard to predict and are likely to occur non-linearly (Seager et al. 2007).

As an example, drought and climate change will cause changes in fire regimes in many vegetation communities. The timing, frequency, extent, and destructiveness of wildfires are likely to increase and may facilitate the invasion and increase of nonindigenous plants. These changed fire regimes will alter vegetation communities, the hydrological cycle, and nutrient cycling in affected watersheds (Brown et al. 2004). Some regional analyses conservatively predict that acreage burned annually will double with climate change (MacKenzie et al. 2004). Such watershed impacts could cause enhanced scouring and sediment deposition, more extreme flooding (quicker and higher peak flows), and changes to water quality due to increases in ash and sediment within stream channels. Severe watershed impacts such as these, when added to reductions in extant aquatic habitats, will severely restrict sites available for the conservation of native fish and other aquatic vertebrates and make management of extant sites more difficult.

Many of the predictions about the impacts of climate change are based on modeling, but many predictions have already occurred. The tree die-offs and fires that have occurred in the south-west early in this century show the impacts of the current drought. Because of drought, climate change, and human population growth, negative effects to aquatic habitat in the range of the desert pupfish continue to occur. The basin's rivers, streams, and springs continue to be degraded, or lost entirely.

Therefore, while it appears reasonable to assume that desert pupfish may be affected by climate change, we lack sufficient certainty to know how climate change specifically will affect desert pupfish beyond loss, reduction, and degradation of habitat.

## 2.4 Synthesis

The natural populations of desert pupfish are the same as those identified in the 1993 recovery plan (USFWS 1993), with the exception of the addition of the natural population in the wash by Hot Mineral Spa, California. The numbers of pupfish in these sites have waxed and waned, as have the populations of nonnative aquatic species there. Recent releases of *C. m. macularius* have been made in Arizona, but we are not certain if pupfish will establish viable populations (Robinson 2009).

Although the recovery criteria have not been achieved (see Section 2.2.3), progress has been made on implementation of recovery plan tasks (Desert Fishes Team 2006, USFWS 2010b). Several recovery plan tasks have been completed and many more are being implemented and are ongoing (USFWS 2010b). Significant accomplishments include creating refuges in both Mexico and the United States for *C. m. eremus*, attempting to reestablish *C. m. macularius* in Arizona, implementing the Safe Harbor Agreement in Arizona, and assessing the genetic status of all desert pupfish populations (Echelle et al. 2007, Loftis 2007, Koike et al. 2008, Loftis et al. 2009).

However, the threats identified at the time of listing and in the recovery plan continue unabated. New nonnative aquatic species continue to establish within the desert pupfish's range, and previously existing nonnative species increase in numbers and distribution (Minckley and Marsh 2009). Human demands for water are unending, with the Salton Sea, Quitobaquito Springs, and the Rio Sonoyta suffering water level declines and the associated threats to the desert pupfish from water depletion, such as habitat loss, fragmentation, and degradation of habitat quality still ongoing. Water availability to the desert pupfish will continue to interact with predicted trends for warmer, drier, and more extreme hydrological conditions associated with climate change.

Work on the genetics and taxonomy of *C. macularius* has led to the division of the taxon into three species. This has effectively reduced the historical range of *C. macularius.* However, because *C. arcuatus* is likely extinct and is also considered ecologically similar to *C. macularius*, the range of *C. arcuatus* in the Santa Cruz River basin will be stocked with *C. macularius*.

## 3.0 RESULTS

### 3.1 Recommended Classification:

**_____ Downlist to Threatened**
**_____ Uplist to Endangered**
**_____ Delist**
____ *Extinction*
____ *Recovery*
____ *Original data for classification in error*
__X__ **No change is needed**

### 3.2 New Recovery Priority Number: No change, remain as 2C.

**Brief Rationale:** The threats and their overall level of intensity remain similar to when the species was originally given a recovery priority number of 2C. A 2C is indicative of a high degree of threat, a high potential for recovery, and the listed entity (or entities) is a (are) species. Conflicts over water still remain and are likely to exist in the foreseeable future.

### 3.3 Listing and Reclassification Priority Number: N/A.

## 4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

- The Desert Pupfish Recovery Plan should be revised to incorporate taxonomic changes of the desert pupfish and updated genetic and management protocols.
  - A specific genetic protocol should be developed, using work by Echelle et al (2007) as a template for management of *C. m. macularius and C. m. eremus* refuge populations. Their recommendations include establishing at least four large primary refuge populations, with each one representing one of the four groups of wild *C. m. macularius and C. m. eremus.* The primary refuge populations would receive periodic supplementation with wild fish. They also recommend that 10 or more secondary refuges representing each of the four wild source regions be established. Their report contains additional recommendations on management of the refuge populations (Echelle et al. 2007) that would be of great use in developing an updated, standardized protocol.
  - A recovery plan amendment or revision is also indicated based on recommendations by Loftis et al (2009) that delineate a different set of management units in the Salton Sea than is recognized in the existing recovery plan, and to reflect the changed taxonomy.

- A technical correction should be published in the Federal Register to update the List of Endangered and Threatened Wildlife to include three taxa, the Quitobaquito Springs pupfish (*C. eremus*), the desert pupfish, (*C. macularius*), and the Santa Cruz pupfish (*C. arcuatus*). Such an action will ensure that recognition and protection under the Endangered Species Act is provided for all species equivalent to the originally listed taxon. Even though *C. arcuatus* appears to be extinct, it is possible that a captive population exists somewhere within its historical range of the Tucson basin.

- Develop at least four Refuge Ponds in San Luis, Sonora (and vicinity) for desert pupfish from Cienega de Santa Clara.

- Emphasize conservation at wild sites, where progress can be made, and use Safe Harbor Agreements only where no other progress can be made.

- Continue to emphasize enrollment of large sites under Safe Harbor Agreements, ensuring genetic integrity is maintained and adequate numbers are available for other conservation activities.

- Pursue a Safe Harbor Agreement or similar tool for the desert pupfish in California.

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## Appendix

**Fish species codes**

| | | |
|---|---|---|
| AGCH | *Agosia chrysogaster* | longfin dace |
| CYCA | *Cyprinus carpio* | common carp |
| CYLU | *Cyprinella lutrensis* | red shiner |
| CYMA | *Cyprinodon macularius* | desert pupfish |
| CYMAER | *Cyprinodon m. eremus* | Quitobaquito (=Sonoyta) pupfish |
| CYMAMA | *C. m. macularius* | desert pupfish |
| GAAF | *Gambusia affinis* | western mosquitofish |
| GIMI | *Gillichthys mirabilis* | Longjaw mudsucker |
| ICNA | *Ictalurus natalis* | black bullhead |
| LECY | *Lepomis cyanellus* | green sunfish |
| *Lepomis* | *Lepomis* sp. | sunfish |
| MISA | *Micropterus salmoides* | largemouth bass |
| MUCE | *Mugil cephalus* | striped mullet |
| POLA | *Poecilia latipinna* | sailfin molly |
| TIZI | *Tilapia zilli* | redbelly (=Zill's) tilapia |

**U.S. FISH AND WILDLIFE SERVICE**
**5 - YEAR REVIEW OF DESERT PUPFISH**

**Current Classification:**

**Recommendation resulting from the 5-Year Review:**

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

**Appropriate Listing/Reclassification Priority Number, if applicable:**

**Review Conducted By:**
Doug Duncan, Fish Biologist, Arizona Ecological Services Office – Tucson Sub-office, (520) 670-6150, extension 236; Jason Douglas, Fish and Wildlife Biologist, Arizona Ecological Services Office – Tucson Sub-office, (520) 670-6150, extension 226

**FIELD OFFICE APPROVAL:**

**Lead Field Supervisor, Fish and Wildlife Service**

Approve ______________________ Date 8/30/10

**REGIONAL OFFICE APPROVAL:**

Actg **Lead Assistant Regional Director, Fish and Wildlife Service**

Approve Elizabeth ______________________ Date 9/14/10

**Cooperating Assistant Regional Director, Fish and Wildlife Service**

_X_ Concur ____ Do Not Concur

Signature ______________________ Date 9/29/10