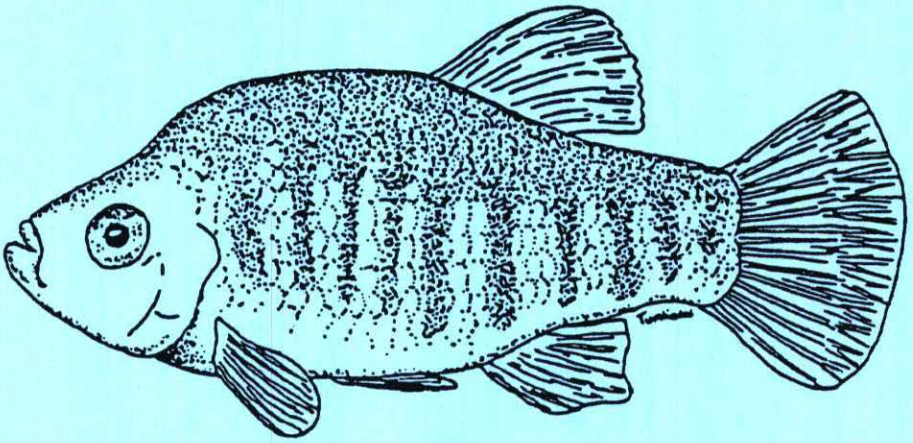
DESERT PUPFISH RECOVERY PLAN



U.S. FISH & WILDLIFE SERVICE

DEPARTMENT OF THE INTERIOR

Phoenix, Arizona

September 1993

DESERT PUPFISH (*Cyprinodon macularius*)

RECOVERY PLAN

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Region 2

U.S. Fish and Wildlife Service
Albuquerque, New Mexico

with assistance from

Arizona Game and Fish Department
and
Tonto National Forest

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Approved: John G. Rogers
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Recovery plans delineate reasonable actions which are believed to be required to recover and/or protect listed species. Plans are published by the U.S. Fish and Wildlife Service, sometimes prepared with the assistance of recovery teams, contractors, State agencies, and others. Objectives will be attained and any necessary funds made available, subject to budgetary and other constraints affecting the parties involved, as well as the need to address other priorities. Recovery plans do not necessarily represent the views nor the official positions or approvals of any individuals or agencies (involved in the plan formulation), other than the U.S. Fish and Wildlife Service. They represent the official position of the U.S. Fish and Wildlife Service only after they have been signed by the Regional Director or Director as approved. Approved recovery plans are subject to modification as dictated by new findings, changes in species status, and the completion of recovery tasks.

LITERATURE CITATIONS

U.S. Fish and Wildlife Service. 1993. Desert Pupfish Recovery Plan. Phoenix, Arizona. 67 pp.

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Fish and Wildlife Reference Service:

5430 Grosvenor Lane, Suite 110
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The fee for the Plan varies depending on the number of pages of the Plan.

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EXECUTIVE SUMMARY

Current Species Status: Listed as endangered throughout its range. Composed of two subspecies in the U.S.: a Colorado River form and a Quitobaquito form. Natural populations of the Colorado River form have been extirpated from Arizona, restricted to three natural locations in California and the non-natural irrigation drains around the Salton Sea. The Colorado River form also occupies certain restricted locations of the Colorado River Delta in Sonora and Baja California, Mexico. The Quitobaquito form persists in a single, modified spring at Organ Pipe Cactus National Monument, Arizona. Distribution of a third, undescribed form in Rio Sonoyta of Sonora, Mexico is unknown, but believed to be quite limited.

Habitat Requirements and Limiting Factors: Cienegas, springs, small streams and margins of large rivers. Has tolerance for wide temperature fluctuation, low oxygen concentrations, and high salinity. Does not cope effectively with introduction of non-native fish. Habitat loss, habitat modification, pollution, and competition and predation from non-native fish threaten the species' survival.

Recovery Objective: Downlisting of the Colorado River form (delisting of Colorado River form is not considered feasible in the foreseeable future), and protection of the other two subspecies (downlisting of Quitobaquito form appears to be unattainable).

Recovery Criteria: Secure, maintain and replicate all naturally occurring extant populations. Re-establish replicate populations in the most natural, identifiable habitats within the probable historical range. Each replicated population will not be considered established until the population has persisted for a minimum of ten years. Protection and establishment of refugium populations of Quitobaquito and Rio Sonoyta forms.

Actions Needed:

1. Protect natural populations and their habitats.
2. Re-establish populations.
3. Establish a refugium population of Quitobaquito pupfish.
4. Develop protocol for exchange of genetic material.
5. Monitor natural and replicated populations.
6. Determine factors affecting population persistence.
7. Information and education.

Costs - (000's):

Year	Need 1*	Need 2	Need 3	Need 4	Need 5	Need 6	Total
1994	26	30	20	8	31	7	122
1995	50	30	20	23	55	6	184
1996	45	25	20	8	56	7	161
1997	36	25	20	10	56	7	154
1998	26	25	20	10	38	7	126
1999-2008	100	150	0	165	40	70	525
Total Costs	283	285	100	224	276	104	1,272

Date of Downlisting: Downlisting is expected to occur in 2009 for the subspecies C. macularius macularius, if downlisting criteria are met.

* - not including acquisition costs.

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I. INTRODUCTION

The desert pupfish (Cyprinodon macularius Baird and Girard) (1853) is a small cyprinodontid fish that once was widespread and abundant in portions of southern Arizona and southeastern California, United States, and northern Baja California and Sonora, Mexico (Miller 1943). Historical habitats varied in size, complexity, character and permanence, and included cienegas, springs, streams, and margins of larger lakes and rivers (Minckley 1973). The desert pupfish has received considerable attention from behaviorists, systematists, physiological ecologists, and geneticists but many aspects of its basic biology remain unstudied. Although remarkably tolerant of extreme environmental conditions (Deacon and Minckley 1974), the species is threatened with extinction throughout its native range primarily because of habitat loss or modification, pollution, and introductions of exotic fishes [U.S. Fish and Wildlife Service (Service) 1986].

Naturally-occurring populations of desert pupfish are now restricted in Arizona to Quitobaquito Springs and in California to two streams tributary to, and a few shoreline pools and irrigation drains of, the Salton Sea. The species is found in Mexico at scattered localities along Rio Sonoyta, on the Colorado River Delta, and in the Laguna Salada basin. The desert pupfish is listed as endangered by the United States (Service 1986), the International Union for Conservation of Nature and Natural Resources (Miller 1979, IUCN 1990), and the States of Arizona [Arizona Game and Fish Department (AGFD) 1988] and California [California Department of Fish and Game (CADFG) 1980, Bolster 1990]. The Mexican government has also listed the species as endangered [Secretaría de Desarrollo Urbano y Ecología (SEDUE) 1991].

Description

The desert pupfish was described by Baird and Girard (1853) from specimens collected in the San Pedro River, Arizona. The taxon now includes two recognized subspecies, Cyprinodon m. macularius and C. m. eremus, and one undiagnosed form which occurs in the Rio Sonoyta, Sonora, Mexico (McMahon and Miller 1985, Miller and Fuiman 1987). Cyprinodon m. eremus is endemic to Quitobaquito Springs, Organ Pipe Cactus National Monument, Pima County, Arizona (Miller and Fuiman 1987). All other populations are referred to C. m. macularius. A third named subspecies, C. m. californiensis (Girard 1859, Miller 1943, Hubbs et al. 1979) from near San Diego, California, is no longer recognized as valid and is now considered C. m. macularius (Miller and Fuiman 1987). Lucania browni Jordan and Richardson (1907) from a hot spring in northeastern Baja California was also synonomized with C. m. macularius (Miller 1943, Minckley 1973, Miller and Fuiman 1987).

Analysis of allozyme variation (Turner 1983) of six desert pupfish populations [Quitobaquito Spring, Boyce Thompson Arboretum (progeny of fish from Cienaga de Santa Clara, Mexico) (=Santa Clara Slough) and four from the Salton Sink] showed mean heterozygosity values within the range reported by Kornfield and Nevo (1976) for the ecologically comparable (Miller 1981) euryhaline killifish Aphanius dispar. The study also detected differences among the three geographic areas and among the four Salton Sink populations, and a low level of inter-population differentiation.

A description of Cyprinodon macularius is summarized from Baird and Girard (1853), Miller (1943), Minckley (1973), and Moyle (1976):

> The body is thickened, chubby or markedly compressed laterally in adult males. The mouth is superior and highly protractile, and is equipped with tricuspid jaw teeth. Spine-like projections are characteristic of scale circuli. The dorsal profile is smoothly rounded.

> Background coloration is silvery in females and juveniles. The sides have narrow, vertical dark bars interrupted laterally and giving an appearance of a disjunct lateral band. Fins are colorless except for a dark ocellus in the dorsal and (rarely) a dark spot on the anal fin. Mature males in breeding condition are brightly colored with the caudal fin and posterior portion of the caudal peduncle yellow or orange, sometimes intense orange-red. Other fins are dark. The body is iridescent light-to-sky blue, especially on the dorsal surface of the head and predorsal region.

The pupfish endemic to Quitobaquito Spring, Arizona, has been long recognized as a distinct form (Miller 1943, Hubbs and Miller 1948, Cole 1963, Cole and Whiteside 1965, Minckley 1973) but not formally described until recently (Miller and Fuiman 1987). The Quitobaquito pupfish (*Cyprinodon macularius eremus*) differs from other populations of *C. macularius* primarily as follows (Miller and Fuiman 1987):

> The males have a longer, wider and deeper head, and broader and deeper body. Distances from the tip of the snout to the pelvic fin insertion, and from snout to anal fin insertion are greater in males. In females, the head is deeper, the body is slightly deeper, the dorsal fin base is longer, and the depressed anal fin is shorter. The dorsal fin origin is more posterior than for typical *C. macularius*, and is the same for males and females. Pelvic fins are reduced in size (as they are in other Rio Sonoyta populations) compared to most *C. macularius*.

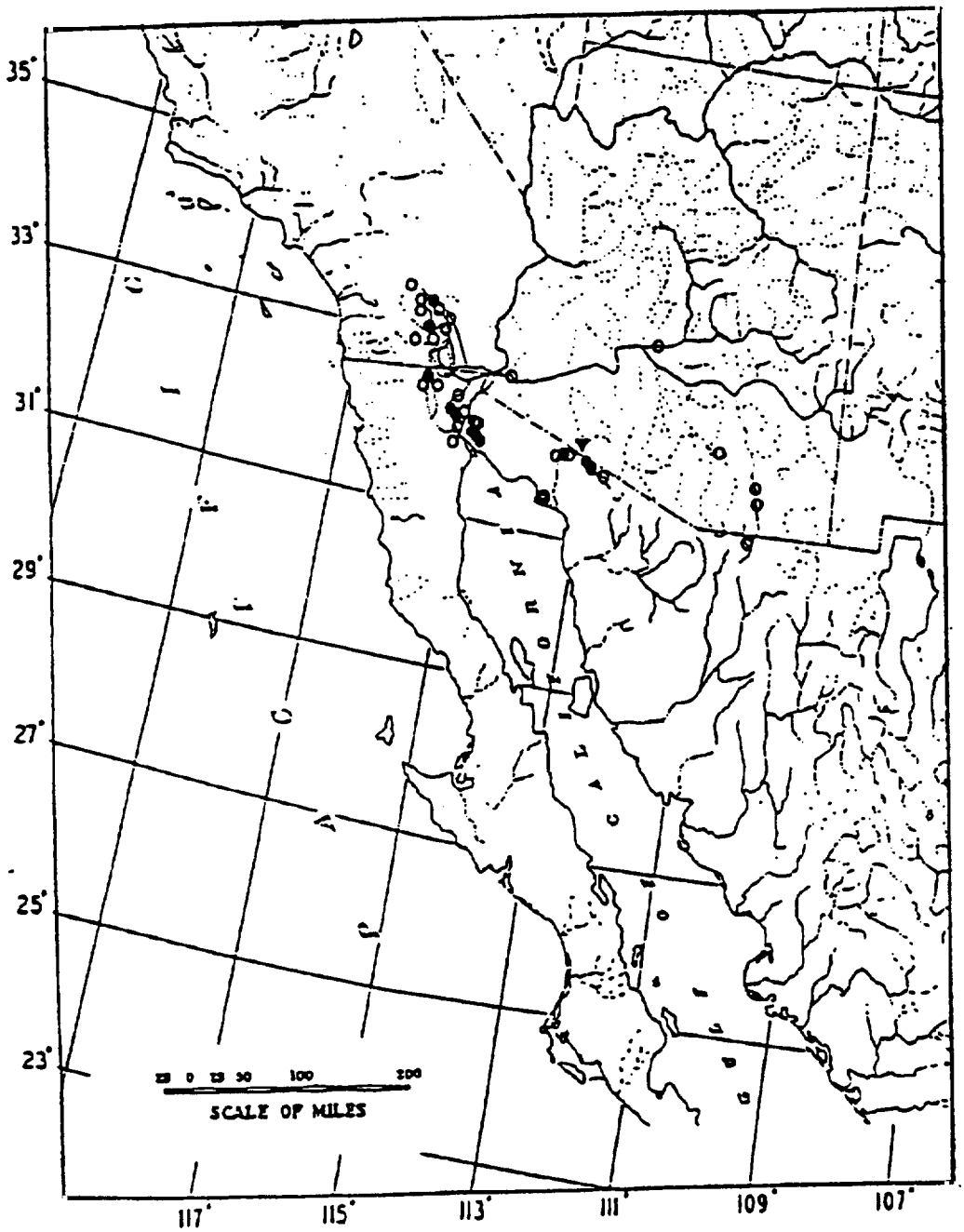
McMahon and Miller (1985) and R.R. Miller (in Minckley 1985) concluded that pupfish from the mainstream Rio Sonoyta differ substantially from those in Quitobaquito Spring, although not at more than a subspecific level. Miller and Fuiman (1987) further note the distinctiveness of Rio Sonoyta populations compared with Quitobaquito pupfish and considered the former an intermediate link between *C. m. macularius* and *C. m. eremus*.

Distribution and Abundance

Historical. Desert pupfish historically occupied the Gila River basin below about 1,500 meters (m) elevation in Arizona and Sonora, including the Gila, Santa Cruz, San Pedro, and Salt Rivers; the lower Colorado River in Arizona and California downstream from the vicinity of Needles to the Gulf of California and onto its delta in Sonora and Baja California; the Rio Sonoyta of Arizona and Sonora; Puerto Penasco, Sonora; and the endorheic Laguna Salada basin of Baja California (Figure 1) (Minckley 1973, 1980; Miller and Fuiman 1987; Miller written communication 1993). Although collections are wanting, suitable habitat was available and the species probably occurred as well in the Agua Fria, Hassayampa, and Verde Rivers of Arizona. In California, it historically occurred in springs, seeps and slow-moving streams in the Salton Sink basin (Eigenmann and Eigenmann 1888, Evermann 1916, Thompson 1920, Jordan 1924, Coleman 1929, Jaeger 1938, Miller 1943, Black 1980b), and possibly in the slow-moving waters along the lower Colorado River (Garman 1895, Gilbert and Scofield 1898, Turner 1983). The Quitobaquito form occurred naturally only in Quitobaquito Spring, Arizona. Historic collection localities are provided in Figure 1.

Distribution of desert pupfish was widespread but probably not continuous within its historic range. Populations occupying stable springs and headwater habitats may have persisted for millennia and experienced relatively little long-term change in numbers. Those occupying rivers and adjacent habitats almost certainly varied numerically in response to local climatic and habitat

Figure 1. Historic collections and present distribution of desert pupfish; open circles represent historic records, closed circles denote extant natural populations of Cyprinodon macularius macularius, and the triangle locates Quitobaquito Spring (Cyprinodon macularius eremus).



conditions. Small populations were found in small habitats and elsewhere during harsh conditions, with expansion into larger habitats when environmental conditions moderated. Populations of larger streams and rivers likely were ephemeral, perishing when drought desiccated their habitat, and dispersing to populate areas watered by flooding. Such a scenario, when repeated over the evolutionary history of the species, would likely have led to panmixia among populations within broad geographic areas.

After the Salton Sink was most recently flooded in the early 1900s by diversion of the Colorado River, desert pupfish colonized what is now known as the Salton Sea (Thompson and Bryant 1920). The Salton Sea, its tributary streams and irrigation drains, supported large desert pupfish populations until precipitous population declines, attributed especially to introductions of exotic species (Miller and Fuiman 1987, Schoenherr 1988) began in the early 1960s (Black 1980b).

Historic abundance of pupfish at Quitobaquito remains unknown because the habitat has been modified by impoundment and diversion by humans (Bryan 1925, Johnson et al. 1983). Habitat likely was relatively small under pristine conditions, and areal densities of fish probably varied little other than seasonally under natural conditions.

Present. Natural populations of the Colorado River subspecies of desert pupfish persist in at least a dozen locales in the United States and Mexico (Fig. 1; Table 1, Appendix), and at least 20 and up to 24 transplanted (non-aquarium) populations are extant (AGFD files; Bagley et al. 1991, Brown and Abarca 1992, Table 2, Appendix). Among the last is a large stock derived from Cienaga de Santa Clara and maintained at Dexter National Fish Hatchery, New Mexico. Quitobaquito pupfish are in its single native habitat (Fig. 1), one population of known genetic purity is established at Arizona State University, several potentially mixed stocks exist (Table 2), and a number of display or aquarium stocks are extant (AGFD files).

Arizona. Naturally occurring populations of *Cyprinodon macularius macularius* have been extirpated from Arizona. However, the subspecies has been transplanted from Dexter National Fish Hatchery (Cienaga de Santa Clara origin) to a number of locations within the state (Table 2). Transplant sites included natural habitats, livestock watering tanks, constructed refugia, and aquaria under State, Federal, or private ownership. At least 8 and as many as 12 Arizona transplant locations supported pupfish in spring 1991, with population sizes of more than 1,000 individuals (Table 2).

A large population of *Cyprinodon m. eremus* is endemic to Quitobaquito Springs, Organ Pipe Cactus National Monument (Fig. 1). Total estimated abundance in the 0.22 hectare (Fisher 1989) pond varies annually from about 5,000 to 10,000 under normal conditions [Kynard and Garrett 1979, Bagley et al. 1991, Brown and Abarca 1992, U.S. National Park Service (NPS) 1992]. A captive stock of Quitobaquito pupfish is currently held at Arizona State University in Tempe (Table 2).

Other populations presumably derived from Quitobaquito Spring, but of questionable genetic purity because of potential genetic contamination by other species or subspecies, were established and may persist at Bog Hole Tank (Coronado National Forest, Santa Cruz County), Finley Tank (Audubon Society Research Ranch near Elgin, Santa Cruz County), Arizona-Sonora Desert Museum (near Tucson, Pima County), and Tohono Chul Park, Tucson (Table 2). These populations should be destroyed because they all are outside the historic range of the subspecies, are of questionable genetic purity, and threaten recovery of downstream populations.

California. Natural populations of desert pupfish are presently restricted in California to San Felipe Creek and its associated wetland, San

Table 1. Summary of known natural populations now existing in the United States and Mexico.

Cyprinodon macularius eremus

1) Quitobaquito Springs, Arizona

Cyprinodon macularius macularius

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

2) El Doctor (3 localities) and Cienaga de Santa Clara (2 localities);

3) Laguna Salada, Baja California; and

4) Cerro Prieto (2 localities), Baja California, Mexico

Cyprinodon macularius ssp.

1) Rio Sonoyta, Sonora

Table 2. Summary of extant transplanted stocks of desert pupfish. Records from spring 1991 unless otherwise designated. Included are location, ownership, transplant date(s), habitat type, approximate population size, and original source of fish (AGFD and CADFG files).

Cyprinodon macularius macularius

1. AZ, Maricopa Co., Tempe; private (W.L. Minckley); 1976, 1988; artificial (concrete) pond; <500 fish; Santa Clara Slough, Mexico.

2. AZ, Graham Co., Howard Well; U.S. Bureau of Land Management; 1983; artificial stock tank supplied by drilled artesian well; status uncertain as of March 1993; Santa Clara Slough, Mexico.

3. AZ, Maricopa Co., Glendale, Deer Valley High School; Glendale School District; 1983, 1987; 1991; artificial (earthen) pond; >500 fish; Santa Clara Slough, Mexico.

4. AZ, Pinal Co., Boyce Thompson Arboretum; University of Arizona; 1983, 1984, 1985; artificial (earthen) impoundment supplied in part by treated sewage and mine water; >500 fish (contaminated by fathead minnow); Santa Clara Slough, Mexico.

5. NM, Chavez Co., Dexter, National Fish Hatchery; U.S. Fish and Wildlife Service; 1983; artificial (earthen) pond supplied by well water; >500 fish; Santa Clara Slough, Mexico.

6. AZ, Pima Co., Tucson, Flowing Wells Junior High School; Tucson School District; 1986; artificial (concrete) pond; <500 fish; Santa Clara Slough, Mexico.

7. Mexico, Sonora, Hermosillo, Centro Ecológico de Sonora; State of Sonora; 1986; artificial pond; >1,000 fish; Rio Sonoyta, Mexico.

8. AZ, Graham Co., Roper Lake State Park; State of Arizona; 1987; artificial (earthen) pond supplied by spring water; status uncertain as of March 1993; Santa Clara Slough, Mexico.

9. AZ, Maricopa Co., Phoenix, Desert Botanical Garden; private; 1987; artificial (concrete) pond; <500 fish; Santa Clara Slough, Mexico.

10. AZ, Pima Co., Buehman Canyon; State of Arizona; 1989; natural, perennial stream; status uncertain as of March 1993; Santa Clara Slough, Mexico.

11. AZ, Maricopa Co., Hassayampa River Preserve; The Nature Conservancy; 1989; artificial (earthen) impoundment supplied by quasi-natural (modified) spring; status uncertain as of March 1993; Santa Clara Slough, Mexico.

12. AZ, Maricopa Co., Glendale; private (R.Engle-Wilson); 1989; artificial (concrete) pond; <500 fish; Santa Clara Slough, Mexico.

13. AZ, Pima Co., Tucson, Arizona Historical Society; private; 1989; artificial (concrete) pond; unknown number of fish; Santa Clara Slough, Mexico.

14. AZ, Graham Co., Cold Spring Seep; BLM; 1990; artificial impoundment (2 small pools); status uncertain as of March 1993; stocked with 50 fish from Flowing Wells Jr. High School, Tucson, and 150 fish from Dexter NFH (both Santa Clara Slough stock).

15. CA, Riverside Co., The Living Desert; private ownership; 1972; two artificial (concrete) ponds; current number unknown; Salton Sea, California.

16. CA, San Diego Co., Palm Spring, Anza-Borrego Desert State Park; 1978; State of California; artificial (concrete) pond, current number unknown; Salton Sea, California.

17. CA, San Diego Co., Visitor Center, Anza-Borrego State Park; 1979; State of California; artificial (concrete) pond, current number unknown; Salton Sea, California.

18. CA, Riverside Co., Oasis Spring Ecological Reserve; 1977, 1979; State of California; artesian well and two earthen ponds; current number unknown; Salton Sea, California.

19. CA, Riverside Co., Salton Sea State Recreation Area; 1982; State of California; artificial (concrete) pond; current number unknown; Salton Sea, California.

20. CA, Riverside Co., Simone/McCallum Pond, Thousand Palms Oasis; 1987; private (The Nature Conservancy); natural spring/artificial (earthen) pond; current number unknown; Salt Creek, California.

21. CA, Riverside Co., Visitor Center Pond, Thousand Palms Oasis; 1989; private (The Nature Conservancy); natural spring/artificial (earthen) pond; current number unknown; Salt Creek, California.

22. CA, Riverside Co., Rancho Dos Palmas; 1990; private (BLM); artificial (earthen) pond; current number unknown; Salt Creek, California.

23. CA, San Diego Co., Palm Canyon, Anza-Borrego Desert State Park; State of California; 1981; artificial (concrete) pond; current number unknown; San Felipe Creek, California.

24. CA, Riverside Co., The Living Desert; private ownership; 1985, 1987; artificial (earthen) pond; current number unknown; San Felipe Creek, California.

25. Numerous captive aquarium populations (See Appendix).

<u>Cyprinodon</u> <u>macularius</u> <u>eremus</u>, including stocks of questionable genetic purity.

1. AZ, Santa Cruz Co., Bog Hole; U.S. Forest Service; 1977; artificial (earthen) impoundment on natural drainage; < 500 fish; potentially mixed stocks.

2. AZ, Santa Cruz Co., Finley Tank; Audubon Society; 1978; artificial (earthen) impoundment fed by springwater; >500 fish; potentially mixed stocks.

3. AZ, Pima Co., Tucson, Arizona-Sonora Desert Museum; private; 1981; artificial (concrete) ponds; >500 fish; potentially mixed stocks.

4. AZ , Pima Co., Tucson, Tohono Chul; private; 1987; artificial (concrete) pond; <500 fish; potentially mixed stocks.

5. AZ, Maricopa Co., Tempe; Arizona State University; State of Arizona; 1989; artificial (concrete) pond; >500 fish; Quitobaquito Springs (Organ Pipe Cactus National Monument), Arizona, via Arizona Game and Fish Department.

6. Numerous captive aquarium populations (See Appendix, AGFD files).

Sebastian Marsh, Imperial County; upper Salt Creek, Riverside County; and a few isolated shoreline pools and irrigation drains along the Salton Sea, Imperial and Riverside Counties., (Fig. 1; Miller and Fuiman 1987, Nichol et al. 1991). Relatively small refugium populations have been transplanted to Arrowweed Spring (Imperial County), Butte County Mosquito Abatement District (Butte County), Rancho Dos Palmas, Salton Sea State Recreation Area, The Living Desert (two populations), Thousand Palms Oasis (two locations), and Oasis Spring Ecological Reserve (Riverside County), and Palm Spring, Palm Canyon, and Visitor Center, located at Anza-Borrego State Park in San Diego County (Table 2).

Mexico. Natural populations of the yet-undescribed form of desert pupfish persist in Sonora in Rio Sonoyta (Fig. 1). *Cyprinodon m. macularius* is in several spring-fed marshes in the vicinity of ~~the village of El Doctor~~ and in Cienega de Santa Clara, Sonora. Desert pupfish in Baja California are found on the Colorado Delta, in Laguna Salada, in an expansive wetland associated with a geothermal powerplant at Cerro Prieto, and in a ditch downstream of the Cerro Prieto marshland (Fig. 1; Hendrickson and Varela-Romero 1989). A captive population of pupfish from the Rio Sonoyta was established at Centro Ecológico de Sonora (CES) in Hermosillo, but a stock obtained from Santa Clara Slough and also held there was recently extirpated. There are no other records of desert pupfish transplants within Mexico.

Life History

Research on desert pupfish has included study of taxonomy and biogeography (Miller 1943 and 1981, Hubbs and Miller 1948, Miller and Fuiman 1987, Hendrickson and Varela-Romero 1989, others), physiology (e.g., Barlow 1958a, Kinne 1960, Kinne and Kinne 1962a and b, Sweet and Kinne 1964, Lowe et al. 1967, Courtois and Hino 1979, Schoenherr and Feldmeth 1991), genetics (e.g., Turner 1983 and 1984; Echelle 1991, Echelle and Dowling 1992, Echelle and Echelle 1993), and behavioral ecology (e.g., Cowles 1934, Barlow 1958b and 1961; Arnold 1972, Loiselle 1980 and 1982, Matsui 1981, McMahon 1984, McMahon and Tash 1988). Because of this broad spectrum of examination, the desert pupfish may be the best known member of the cyprinodontid family of fishes.

Habitat. Desert pupfish occupied a diversity of habitats ranging from cienagas and springs to small streams and margins of larger bodies of waters. Most habitats were shallow and had soft substrates and clear water. Abundance of aquatic vegetation and invertebrates probably varied seasonally, with lowest levels associated with harshest conditions.

Pupfish have an extraordinary ability to survive under conditions of high water temperature (to 45°C, Lowe et al. 1967), low dissolved oxygen concentration [0.1-0.4 milligrams per liter (mg/L), (Barlow 1958b)], and high salinity [salt concentrations twice (68 grams per liter) that of seawater, Lowe et al. 1967], which exceed tolerances of virtually all other freshwater fishes (see also Kinne 1960, Kinne and Kinne 1962 a,b). They also survive abrupt, absolute changes in both salinity [10-15 grams per liter (gm/L)] and temperature (22-26°C) (Kinne 1960, Lowe and Heath 1969) that are lethal to most fishes. In less harsh environments where a greater diversity of fishes was found (e.g., margins of larger streams and rivers), pupfish typically occupied water shallower than that inhabited by adults of most other species.

Reproduction. Under conditions of abundant food and suitable temperature (mid-to-upper 20s °C), desert pupfish may become sexually mature as early as six weeks of age at 1.5 centimeters (cm) total length (Moyle 1976). Although they may breed during their first summer, most do not breed until their second summer, when their length may have reached a maximum of 7.5 cm (Moyle 1976). Male pupfish are usually highly aggressive during the breeding season (early spring into winter when water temperature exceeds about 20°C). During this period they establish, actively patrol, and defend individual territories that

are typically in water less than 1 m deep and associated with a small structure or incongruity on the substrate (Barlow 1961). Males in natural habitats normally defend 1 to 2 square meters of bottom, depending on their individual size, density of other male pupfish, and water temperature (Moyle 1976). Minimum male territory size may be 45 to 60 square cm, the density at which population stability is achieved in aquaria (Minckley 1973).

Male breeding behaviors include territoriality and consort pair breeding (a non-territorial system in which males show low levels of aggression) (Kodric-Brown 1981). Territoriality occurs in large habitats with high primary productivity, limited breeding substrates, and high population densities. Consort-pair breeding is characteristic of populations in habitats of low primary productivity, low population density, and abundant breeding habitat (Kodric-Brown 1981). Because territoriality is the most common breeding system in desert pupfish (Barlow 1958b and 1961; Cox 1966, Kodric-Brown 1981), it is further described below.

Adult females swim in loose schools and forage inconspicuously. A female that is ready to spawn leaves the school when attracted by a territorial male (Cowles 1934, Barlow 1961). As the two fish move toward one another, the female tilts head-first toward the bottom and takes a small piece of substrate into her mouth. After resuming a horizontal position, she spits out the material. This sequence may be repeated several times until she ceases motion near the bottom. The male then assumes a position against and parallel to the female, and the two fish contort together to form an "S" shape. The male's anal fin next cups around the vent region of the female, and she vibrates and produces a single, relatively large [ca. 2 millimeters (mm) diameter (Constanz 1981)] egg, which is immediately fertilized. The spawning act takes less than a minute but may be repeated in quick succession to deposit several eggs. In the laboratory, female pupfish of varying size may lay 50 to more than 800 eggs in a single season (Crear and Haydock 1971). Eggs appear to be randomly deposited within the male territory and there is no directed parental care. However, male activities within the territory effectively exclude other fishes, which may enhance chances for successful incubation (Minckley 1973). Incubation time varies with water temperature, hatching in the laboratory occurs in about 10 days at 20°C (Crear and Haydock 1971).

Growth. Growth rate is dependent upon age, habitat and environmental conditions, and population density. In the laboratory, young fish derived from the Salton Sea population exhibited optima growth at 30°C and 35 gm/L salinity, while older individuals grew most rapidly at 22 to 26°C and about 15 gm/L salinity (Kinne 1960, Kinne and Kinne 1962a, b). Body shape varied among fish incubated at different combinations of salinity and temperature (Sweet and Kinne 1964). Temperature effects on size at hatch at constant (35 gm/L) salinity were interpreted to reflect temperature and possible salinity optima for utilization of yolk by developing embryos (Blaxter 1969).

Desert pupfish from the Salton Sea hatch at 0.4-0.5 cm total length and may double in length within the first 8 weeks of life. Depending primarily upon temperature, size ranges from 1.5 to 2.8 cm at 24 weeks of age, and lengths of 4.5 to 5.0 cm are attained in the laboratory by the end of the first growing season (Kinne 1960). Maximum length [to 7.5 cm (Moyle 1976)] may be attained by the second summer. Quitobaquito pupfish in June averaged 29.6 mm at age 1, 40.2 mm at age 2, and 48 mm at age 3 (Kynard and Garret 1979).

Life span in the wild appears highly variable; from less than a year for some populations (Minckley 1973), two years for others (Moyle 1976), and up to three years for Quitobaquito pupfish (Kynard and Garrett 1979). Predation by aquatic insects, piscivorous birds, and mammals was noted by Cole and Whiteside (1965) in Quitobaquito Spring and likely is a source of mortality elsewhere (see Walker 1961).

Foods and Feeding Habits. Larval pupfish in the laboratory begin feeding on tiny invertebrates within a few hours to a day after hatching (Crear and Haydock 1971) and presumably do so in the wild as well. As they grow, wild fish become opportunistic omnivores, consuming whatever variety of algae, plants, suitably-sized invertebrates, and detritus is available (Cox 1966 and 1972, Naiman 1979). Adult foods include ostracods, copepods, and other crustaceans and insects, pile worms, molluscs, and bits of aquatic macrophytes torn from available tissues. Detritus or algae are often predominant in their diets. Pupfish at Quitobaquito Spring have been reported to eat their own eggs and young (Cox 1972), and it has been suggested (Loiselle 1980) that males differentially consume eggs within their territories that were fertilized by other males. Pit digging, the active excavation of soft bottoms in search of foods, is a pupfish behavior described in detail by Minckley and Arnold (1969); these pits are defended when occupied. Foraging is typically a daytime activity, and fish may move in response to daily warming from shallower water during morning to feed in deeper places later in the day.

Co-occurring Native Fishes. The harshest habitats historically occupied by desert pupfish had temperatures, salinities, and dissolved oxygen concentrations so extreme that other fishes were excluded. Elsewhere in cienegas, springs, and small streams, the Sonoran topminnow (*Poeciliopsis occidentalis*) was a common co-habitant; however, it is unknown how the two species interacted. Topminnows and pupfish also inhabited the margins of larger rivers, where shallow depths, high temperatures, or other factors excluded adults of most species. Other fishes in desert pupfish habitats included Gila chub (*Gila intermedia*), speckled dace (*Rhinichthys osculus*) and the desert sucker (*Pantosteus clarki*), but these typically inhabited deeper waters and presumably had little interaction with pupfish. Longfin dace (*Agosia chrysogaster*), Sonora sucker (*Catostomus insignis*), and roundtail chub (*Gila robusta*) were commonly found in mainstream and deeper portions of mid-sized streams occupied peripherally by pupfish. Bonytail (*Gila elegans*), razorback sucker (*Xyrauchen texanus*), Colorado squawfish (*Ptychocheilus lucius*), and woundfin (*Plagopterus argentissimus*) occupied the mainstream of larger rivers. It is doubtful there was opportunity for these species, except as larvae or early juveniles, to interact with pupfish. Longfin dace was the only native fish with potential to have co-occurred with pupfish at Quitobaquito Spring (Minckley 1973).

Reasons for Decline

There are many reasons for declines of desert pupfish populations. They include habitat loss (dewatering of springs, some headwaters, and lower portions of major streams and marshlands), habitat modification (stream impoundment, channelization, diversion, and regulation of discharge, plus domestic livestock grazing and other watershed uses such as timber harvest, mining, and road construction), pollution, and interactions with non-native species (competition for food and space, and predation) (Matsui 1981, Minckley 1985, Service 1986, Miller and Fuiman 1987).

Many historic pupfish localities have been dried by groundwater pumping (affecting both spring and stream discharges), channel erosion or arroyo formation (resulting in drainage of marshlands, creation of sheer banks, and loss of lateral habitat), and water impoundment and diversion (reducing or eliminating stream flows and natural flow regimes) (Hastings and Turner 1965, Fradkin 1981, Rea 1983, Hendrickson and Minckley 1985). Impoundment also creates upstream habitat unsuitable for pupfish because of increased depth and which, because of its lentic character, is more conducive to occupation by non-native fishes. Poor grazing practices by domestic livestock may reduce terrestrial vegetative cover, enhance watershed erosion, exacerbate problems of arroyo cutting, and increase sediment loads and turbidity in receiving waters. Habitats may be further impacted by trampling where cattle feed or drink in or adjacent to water.

Fishes now occupying former desert pupfish habitat include many non-native species (see Miller 1961, Minckley 1973, 1979a and b, Moyle 1976, Marsh and Minckley 1987). These fishes pose the greatest threat to extant desert pupfish populations (Minckley and Deacon 1968, Deacon and Minckley 1974, Schoenherr 1981 and 1988, Meffe 1985, Miller and Fuiman 1987). Pupfish do not fare well in the presence of non-native fishes and incursions by exotics have typically resulted in decline or extirpation of pupfish. Non-native fishes that occupy habitats also used by pupfish [e.g., adult western mosquitofish (Gambusia affinis), sailfin molly (Poecilia latipinna), largemouth bass (Micropterus salmoides), and juvenile cichlids (Oreochromis ssp. and Tilapia ssp.)] have proven most destructive to populations of native species. Primary mechanisms of replacement include predation and aggression (mosquitofish and largemouth bass) and behavioral activities that interfere with reproduction (mollies and cichlids) (Matsui 1981, Schoenherr 1988).

Interactions with introduced mosquitofish were noted early as contributory to the decline of pupfish in the Salton Sea (Evermann 1930, Jennings 1985). Pupfish populations declined further when sailfin molly and African cichlids became abundant (Schoenherr 1979, 1985, and 1988, Black 1980a and b, Matsui 1981). In the Salton Sink, pupfish survive as remnant populations in tributary streams, a few shoreline pools, and irrigation drains where actual or potential invasion by non-native fishes (i.e., centrarchids, cichlids, ictalurids, and poeciliids), threaten their survival.

The Quitobaquito pupfish was threatened by establishment of golden shiner (Notemigonus crysoleucus) following unauthorized stocking in 1968 or 1969 (Minckley 1973). Eradication of the shiner and re-establishment of the pupfish were costly in time, money, and effort. In addition, an August 5, 1993, memorandum from the Superintendent of Organ Pipe National Monument notified the Service that an unconfirmed species of catfish was discovered in Quitobaquito Spring (written communication, H. Smith, Organ Pipe National Monument). The specimen was later identified as a black bullhead (Amieurus melas) (W.L. Minckley, ASU, pers. comm).

Pupfish populations in Mexico have been impacted by proliferation in recent years of non-native fishes (May 1976, McMahon and Miller 1985, Miller and Fuiman 1987, Hendrickson and Varela-Romero 1989). African cichlids, mosquitofish, sailfin molly, red shiner (Cyprinella lutrensis), carp (Cyprinus carpio), and channel catfish (Ictalurus punctatus) are now widespread on the Colorado River Delta. In Rio Sonoyta, Sonora, former and present pupfish habitats are variously infested with mosquitofish and black bullhead (Amieurus melas).

Non-native bullfrog (Rana catesbeiana) may also prove problematic in the management of desert pupfish. This species was introduced to California early in the 1900s (Storrer 1922) and rapidly became established over a wide geographic range in the West, where it has extirpated or displaced several native amphibians (Clarkson and deVos 1986). The bullfrog is an opportunistic omnivore with a diet throughout its range that includes fish (Frost 1935, Cohen and Howard 1958, Brooks 1964, McCoy 1967, Clarkson and deVos 1986). Its potential for impact on desert pupfish was demonstrated in an artificial pond at Arizona State University, where a population of desert pupfish numbering in the thousands was nearly eliminated by fewer than 20 adult bullfrogs over a period of approximately a year. Natural and re-established populations of desert pupfish may thus be at risk where bullfrogs become established, and their removal may be required to assure viability of the native fish.

Drift from aerial application of pesticides, in proximity to pupfish populations, has contributed to the decline of Quitobaquito pupfish (Kynard 1981, Miller and Fuiman 1987). Aerial pesticide application is a common practice near other natural populations (e.g., Rio Sonoyta, Mexico; lower San Felipe Creek, California and a small portion of the upper creek) which may be similarly impacted.

Elevated concentrations of mercury have been detected in tissue samples from a cichlid fish (Tilapia mossambica) and Asian clam (Corbicula fluminea) collected in the vicinity of the Cerro Prieto geothermal field in Mexico (Gutierrez-Galindo et al. 1988). Although measured levels (maximum in fish of 0.14 micrograms per gram dry weight) were below that considered hazardous to human health, potential acute or chronic effects on aquatic life, including some portion of desert pupfish life cycle, have not been determined.

There is also concern that introduced saltcedar (Tamarisk) adjacent to pupfish habitat may cause a lack of water at critical times (Bolster 1990, R. Bransfield pers. comm.). Evapotranspiration by luxuriant growths of this plant may especially impact smaller habitats where water supply is limited.

II. RECOVERY

Objective

The objective of this recovery action plan is to describe actions necessary to eliminate threats to extant populations and successfully establish additional populations of desert pupfish in secure habitats within probable historic range. Once these actions are successfully completed to fulfill the specific criteria delineated below, downlisting of the Colorado River subspecies of desert pupfish (Cyprinodon macularius macularius) from endangered to threatened status will be considered. Because of insoluble threats and limited habitat, delisting of this subspecies is not considered feasible in the foreseeable future.

Neither down- nor delisting of Quitobaquito pupfish (C. m. eremus) is expected because of its limited range, continuing threats to its survival, and lack of historic range in which the subspecies can be recovered. However, this plan provides specific recovery actions determined necessary to ensure survival of this subspecies.

Downlisting Criteria

Desert pupfish (Cyprinodon macularius macularius) will be considered for downlisting when:

(1) Naturally occurring populations in the United States and Mexico are secure. These include five metapopulations at 12 known locations:
   (a) Salton Sink (San Felipe Creek/San Sebastian Marsh, upper Salt Creek, and shoreline pools and irrigation drains of Salton Sea, California);
   (b) Rio Sonoyta, Sonora;
   (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, Mexico;

(2) Populations of desert pupfish are re-established and secure within probable historic range according to specifications detailed in task 2 of this plan;

(3) A protocol for exchange of genetic material among re-established populations is developed and implemented to ensure maintenance of natural levels of allelic genetic diversity; and

(4) Population and genetic monitoring plans as outlined below in the stepdown of this plan are devised and implemented to routinely assess status of all populations.

Security is herein defined as formal protection of habitat and water rights by methods such as land and water rights acquisition, legislation, or management agreement, and maintenance of a genetically pure, self-sustaining, stable or increasing (viable) population. Until additional information becomes available, a viable population (Lacy 1987, Ryman and Utter 1987, Soulé 1987, Templeton 1990) will include not 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.

In the United States, formal protection of water and land will be considered to occur when one of the following criteria is met.

(1) Water rights and habitat associated with each naturally occurring population are in the legal possession of an agency, or organization, or entity whose goals include protection and recovery of endangered species, which possess adequate statutory authority to protect those populations against other land and water uses which may adversely affect desert pupfish, which has adequate regulations in place to enforce such authority, and which has demonstrated over a period of not less than 10 years adequate capability to protect and manage a viable population of desert pupfish.

(2) A legally-binding, long-term (>25 years) agreement is in place between the land and water rights owner(s) and an agency, organization, or entity such as described above, which provides sufficient legal rights to the agency or organization to manage a viable population of desert pupfish. The efficacy of this agreement should be demonstrated over a period greater than (if not equal to) 10 years.

In Mexico, formal protection of land and water will be considered to occur when security comparable to that defined for the United States is achieved.

Locally adjacent desert pupfish populations are considered separate only if a discrete catastrophic event (e.g., invasion by exotic fishes, habitat destruction, etc.) is likely to impact only one population. Unless demonstrated otherwise on a case-by-case basis, the presence of non-native fishes is considered a threat to desert pupfish population viability.

Once this plan is finalized and approved, downlisting of C. m. macularius is expected to take 15 years. Total recovery (delisting) is not expected in the foreseeable future.

Narrative Outline for Recovery Actions Addressing Threats

Factors considered above continue to threaten existence of desert pupfish populations. Increasing human populations continue to deplete available water resources and impact habitats used by desert pupfish. Although major water development projects in the United States have largely been completed, impoundment, stream diversion and groundwater pumping can be expected to continue and increase in the foreseeable future, both in this country and in Mexico. Habitat alteration and loss resulting from past land management practices continue to occur as damaged watersheds struggle to stabilize. Ongoing dispersed land uses will continue to disrupt that stabilization process with potential adverse effects on aquatic ecosystems. Localized agriculture, mining, recreation, and other activities will thus continue to threaten individual desert pupfish populations. Water pollution resulting from drift of agricultural pesticides may impact populations in both countries as agricultural development expands in Mexico and portions of California. Finally, non-native organisms constitute continuing threats to desert pupfish populations throughout their range because introduced species may have the capability to extirpate pupfish and may also be impractical to eradicate or control.

Desert pupfish recovery will require efforts of private and government agencies and organizations in Arizona, California, Sonora, and Baja California. These include, but are not limited to, the Service Regions 1 and 2, Forest Service Region 3, NPS, Bureau of Land Management (BLM), Centro Ecológico de Sonora (CES), Secretaria de Agricultura y Recursos Hidráulicas (SARH), Secretaría de Desarrollo Social (SEDESOL), AGFD, CADFG, Arizona State Land Department, California State Lands Commission, National Audubon Society, and The Nature Conservancy (TNC). Recovery efforts will be effected by subsets of the above participants, as dictated by political boundaries and management authority. The program herein addresses threats to the species and recovery tasks that are necessary to recover the Colorado River form of the desert pupfish throughout its native range, and maintain Quitobaquito and Rio Sonoyta forms. Management plans developed subsequent to this plan will detail actions specific to each state or population.

Recovery actions in the United States emphasize relatively small habitats and establishment of refugium populations, whereas those in Mexico will be most concerned with protecting marshlands and larger areas occupied by desert pupfish and other native species. However, successful implementation of this recovery plan in both countries is required for recovery of the species.

Progress toward recovery of the desert pupfish has been initiated by numerous agencies and organizations. For example, management plans, programs, or activities that include desert pupfish or target specific populations have been prepared or undertaken by AGFD, Arizona State Lands Department, California Department of Parks and Recreation, CADFG, California State Lands Commission, CES, The Living Desert, TNC, BLM, Service, NPS, and others. Several management plans developed for specific populations identify tasks necessary for their security. Full implementation of tasks described in these and additional plans is necessary to accomplish downlisting criteria defined here.

A hierarchical approach to re-establishment is developed for desert pupfish (task 2, below). The need to maintain the integrity of discrete, naturally-occurring stocks while also recognizing a requirement for exchange of genetic material is vital for recovery. This hierarchical approach accommodates (1) protection of naturally occurring populations, (2) replication of each distinct naturally occurring population with re-established populations in the best available sites, (3) opportunity to conduct genetic exchange within re-established populations, and (4) flexibility in protection of the desert pupfish by maximizing recovery success potential while minimizing probability of catastrophic population loss through tiered population management.

Because extant wild populations of desert pupfish are the most valuable remaining reservoir of original genetic material, their security is the most important consideration. From these, a second tier of populations will be established in the wild in the best available natural habitats, and among which individuals can be exchanged to maintain genetic variability. A third tier of populations would be established in natural or "quasi-natural" refugia. While these third tier habitats might be considered inferior or marginal relative to tier-two habitats, they must nonetheless be suitable for long-term maintenance of desert pupfish. Genetic exchange should occur both among third-tier populations and from second- to third-tier populations but not the reverse (see task 2, below). As new information becomes available, specific exchange protocols will be developed and implemented to enable desert pupfish evolution to occur as naturally as possible.

TASK 1. PROTECT NATURAL POPULATIONS OF DESERT PUPFISH

1.1 Identify Land Ownership of Extant Populations and Natural Habitats

Naturally occurring, wild populations of desert pupfish persist at Quitobaquito Springs, Arizona; two Salton Sink localities (plus shoreline pools and irrigation drains) in California; several localities in Rio Sonoyta, Sonora; and the Colorado River Delta, Sonora and Baja California. Specific private and U.S. or Mexican local, State, or Federal landowners must be accurately identified for all extant pupfish populations. The population at Quitobaquito Spring lies entirely within boundaries of Organ Pipe Cactus National Monument and is thus under control of the U.S. Government. Land within and adjacent to pupfish habitats in California is in a mosaic of private and Federal ownerships. Mexican pupfish habitats are primarily in State or private ownership. Most of the property along Rio Sonoyta and lower Colorado River Delta is under local ejido ownership, while pupfish habitat at Cerro Prieto is privately controlled.

1.2 Acquire Habitats Occupied by Natural Populations of Desert Pupfish.

Desert pupfish and their habitats cannot be protected until land and water rights ownerships are in the hands of entities that will ensure protection of the species and its environs. Special consideration must be paid to acquisition of properties or legal agreements in Sonora and Baja California, Mexico, where substantial pupfish habitat remains unprotected. Appropriate mechanisms must be used to acquire any lands in private ownership where such protection is not expected to be forthcoming. Most pupfish habitats in the United States are already under Federal ownership, or ownership by private parties whose conservation goals include perpetuation of desert pupfish. However, these populations and their habitats are not necessarily secure.

Assurance of an adequate water supply through time must be accomplished on a case-by-case basis. The source of water (e.g., aquifer, local watershed, stream channel, etc.) must first be specifically and accurately determined. In instances where water management adversely affects pupfish habitat (e.g., groundwater mining resulting in water level reduction) appropriate mechanisms must curtail the offending water use. Where long-term impacts to pupfish habitat can be predicted, a plan must be prepared and implemented to ensure an adequate water supply. This could be accomplished by a variety of mechanisms, including water rights acquisition, legal protection of instream flows, land and water use agreements, and improved water and/or land-use practices. Specific mechanisms will be determined on a case-by-case basis for each habitat.

1.3 Secure Natural Populations and Their Habitats.

Once land and water ownership or management title has been acquired, several tasks must be accomplished before desert pupfish in any particular habitat can be considered secure. These include promulgation of regulations which will provide sufficient long-term protection and management (e.g., specific designation as Areas of Critical Environmental Concern, Research Natural Areas, etc.), assurance of water of sufficient quantity and quality, protection against habitat degradation, control or removal of deleterious non-native animals and vegetation (if present), prevention of invasion by non-native fishes, and modification of land management practices deleteriously affecting aquatic habitats. Implementation of specific tasks required to achieve population and habitat security must be directed by individual management plans for each site.

Impacts of activities such as livestock grazing or watering, mining, timber harvest, phreatophyte control, recreation, agricultural or residential

development, etc., must also be determined for each pupfish habitat. Appropriate management plans must be formulated for each site or group of sites and implemented to reduce or eliminate impacts so populations are secure. Populations will be considered secure only when the plan is in force and being implemented properly. The goal is to ensure adequate water and habitat to secure pupfish populations meeting criteria specified above.

Unless information becomes available to the contrary, desert pupfish populations cannot be considered secure in habitats occupied by non-native fishes. Thus, habitats presently occupied by desert pupfish and detrimental non-native fishes must be considered, on a case-by-case basis, for reclamation to remove the non-native(s). Habitats in need of renovation should be ranked in consideration of the following criteria:

(a) Natural populations should be considered the first priority for recovery (as opposed to re-established populations),
(b) Immediacy of the threat of extirpation due to presence of non-native fishes,
(c) Status of populations of the same genetic composition,
(d) Ease of reclamation,
(e) Probability of success,
(f) Security against re-infestation by non-native fishes, and
(g) Other general and site-specific factors

Each operation must be supported by the Service, responsible resource agency(ies), the Desert Fishes Recovery Team, and other affected parties. Each operation should be supported by sufficient personnel, equipment, funding, and expertise to maximize chances for success. Inadequate planning, insufficient support, and lack of follow through are major contributors to past reclamation failures (see, e.g., Marsh and Minckley 1990), and those projects without such support must not be initiated until adequate support is available.

Securing desert pupfish populations also requires protecting the habitat against contamination/re-contamination by non-native fishes. Such assurance must be accomplished on a case-by-case basis, depending upon the specific characteristics of each habitat. Provisions might include construction of barriers to preclude natural invasion from confluent waters, removal of offending fishes from confluent or potentially confluent habitats (e.g., livestock watering tanks), imposition of regulations locally prohibiting possession of non-native fishes, and modifying habitat to exclude non-natives. Where habitat reclamation is required, it is imperative to ensure against reinvasion by non-native fishes before renovation is conducted. Public education about desert pupfish and its plight have obvious benefits.

Non-native bullfrogs may also prove problematic in the management of desert pupfish. The diet of bullfrogs includes fish, and its potential impact on pupfish has been documented. Both natural and re-established populations of desert pupfish may thus be at risk where bullfrogs become established. Control or removal of bullfrogs may be required to assure viability of the native fish.

In addition to threats from non-native species, the desert pupfish also faces threats to genetic integrity from contamination by other species or subspecies of pupfish stocked outside of their historic range. Populations of questionable genetic purity may be present in Arizona in Bog Hole Tank (Coronado National Forest, Santa Cruz County), Finley Tank (Audubon Society Research Ranch near Elgin, Santa Cruz County), Arizona-Sonora Desert Museum (Tucson, Pima County), and Tohono Chul Park (Tucson). These populations are all outside of the historic range of the species and threaten recovery of downstream populations. These sites should be renovated to remove the existing populations.

Other habitat management activities may also be required and must be considered on a case-by-case basis. For example, aquatic and/or terrestrial vegetation control may be required to maintain suitable desert pupfish habitat.

A key feature of desert pupfish conservation in Mexico (CES 1990) is the acquisition and expansion of presently-protected areas to include important habitats along Rio Sonoyta and the lower Colorado River Delta. The Reserva del la Biosfera El Pinacate (Pinacate Reserve) could be expanded to incorporate pupfish habitats in Rio Sonoyta. Similar opportunities exist for protection of desert pupfish and their habitats in the lower Colorado River Delta, where a natural area is protected for conservation of totoaba [*Cynoscion macdonaldi* (Perciformes: Sciaenidae)].

TASK 2. RE-ESTABLISH DESERT PUPFISH POPULATIONS

This plan incorporates a 3-tier plan for protection, re-establishment, and recovery of desert pupfish. Extant natural populations will be designated tier 1, which represent the original genotypes, are recognized as the most valuable resource, and will receive the highest level of protection.

Populations designated tier 2 are replicates of remaining, naturally occurring stocks. Tier 2 will be composed of re-established populations in the most natural (i.e., historic condition) identifiable habitats within probable historic range. Preference will be given to those habitats which are most likely to persist in perpetuity without human intervention. If sufficient sites meeting that criteria are not available, then tier 2 populations will be placed into habitats which are expected to require the least human intervention for maintenance.

A second suite of re-established populations (tier 3) will be in the most-natural habitats remaining after fulfillment of tier 2 requirements (see below). Habitat availability may make it necessary to establish some or all tier 3 populations in "quasi-natural" (i.e., human-modified to imitate historic conditions) sites. Individual tier 3 populations may be lost during the course of recovery management, but the total number specified below is to be maintained continuously. Tier 3 populations will theoretically function to optimize the balance between in- and outbreeding depression. Practically, they insure against loss of existing genetic variation and provide a source of future management opportunities.

Genetic exchange is to be accommodated between tier 2 populations derived from a single natural (tier 1) source, from tier 2 source populations to their tier 3 derivatives (but not the reverse), and between tier 3 populations derived from a single tier 2 source (but not between tier 3 populations from different sources). Continued cooperation with Mexico should allow future acquisition of desert pupfish broodstock. Addition of individuals from existing natural populations (Cienega de Santa Clara, El Doctor) will alleviate problems associated with in- and outbreeding depression which may occur in refugia populations.

Re-established populations in Arizona will be located in the lower and middle Gila (including the Hassayampa and Agua Fria), San Pedro, Santa Cruz, and Salt (including Verde) river drainages. Suitable sites in Mexican portions of the Santa Cruz and San Pedro river drainages should also be considered. Specific sites must be determined by appropriate participating entities, consistent with criteria for potential success of transplanted desert pupfish populations detailed below.

Populations of *Cyprinodon macularius macularius* are to be re-established according to specifications presented in Table 3.

Table 3. Re-establishment specifications for *Cyprinodon macularius macularius* populations.

	Natural Populations	Re-established Populations	
Area	Tier 1	Tier 2	Tier 3
Arizona	0	10	45
California	3	9 (3 reps. of each natural)	27 (9 reps. of each natural)
Colorado Delta	3	9 (3 reps. of each natural)	27 (9 reps. of each natural)
Rio Sonoyta	1	--- 3 of either tier 2 or 3 ---	

Specifications:

Tier 2 populations will receive a high degree of protection and will be long-term populations. A tier 2 population will be considered to be successfully established and count toward recovery if it has survived for 10 years and has required *only* minor management to persist. Minor management may include:

habitat-
1) minor vegetation removal
2) fencing
3) drawing off excess water for wildlife and livestock

populations-
4) population monitoring
5) management for other native species
6) pupfish transfers for genetic maintenance

Major management actions which would preclude a population from being considered successful would include:

habitat-
1) new or modified water supply
2) dredging
3) major vegetation removal
4) habitat (re)construction
5) exotic fish introduction or control

populations-
1) restocking pupfish
2) supplemental stockings of pupfish (for reasons other than genetic protocol)

Tier 3 populations may experience major management activities. Management will not preclude counting populations as contributing towards recovery. The specified total number of populations must be achieved and continuously maintained for 10 years.

Preliminary site determination should be based upon potential habitat suitability for long-term success of a population. Provision of security regarding land ownership, water supply, anti-degradation, and non-native fishes should be addressed secondarily as necessary. The San Pedro River (BLM Riparian National Conservation Area, Cochise County, Arizona) should be considered a priority re-establishment site [as already recommended by Minckley (1987) for desert pupfish plus other extirpated native fishes], because it has high potential and is the type locality for the species. A thorough survey of the upper San Pedro River system, Mexico, should be conducted to determine whether or not a native lineage of desert pupfish remains in that system. If discovered, the population would be the preferred source for downstream re-establishment in the San Pedro river system. Other priority sites should be determined after assessment of potential localities in Arizona, California, and Mexico.

To the extent practicable, efforts should be made to re-establish pupfish into a diversity of habitat types reflective of those occupied historically (e.g., spring, cienaga-marshland, stream, and river margin). Pupfish stocks within each region (Rio Sonoyta, Colorado River Delta, Salton Sea) should be distributed among habitat types, rather than concentrating stocks into a single habitat type.

More than 100 transfers of the Colorado River subspecies of desert pupfish have occurred in Arizona, California, Mexico, and elsewhere, and Quitobaquito pupfish has been stocked or transferred to nearly 30 other locales (Bagley et al. 1991, Brown and Abarca 1992, AGFD files). Although many stockings have failed, at least 30 non-aquarium populations of desert pupfish remain (including several of questionable purity, which must be destroyed). Of 20 populations whose failure was documented in 1989, 8 were due to habitat desiccation, 2 were destroyed by invading exotic fishes, 1 was renovated, and 9 failed for unknown reasons (AGFD files). Although desert pupfish are remarkably tolerant of harsh environmental conditions, there appear to be unknown habitat characteristics that negatively influence pupfish survival. Comparisons among and between habitats that failed for unknown reasons and those remaining could provide valuable information and guidance in selecting transplant sites with the highest probability for long-term success. Any such assessment must be accompanied by careful study of habitats occupied by natural desert pupfish populations. These data should provide a more complete understanding of specific criteria necessary for perpetuation of the species (see task 6, below).

This plan recognizes that an adequate number of unaltered, natural habitats suitable for re-establishment of desert pupfish populations may not exist. In such case, re-construction of suitable habitat meeting necessary criteria should be used to assure that the target number of populations are established.

TASK 3. ESTABLISH A REFUGIUM POPULATION OF QUITOBAQUITO PUPFISH

At least one secure population of the Quitobaquito form must be established in a refugium. This refugium should be located in the vicinity of the species natural range (i.e., Organ Pipe Cactus National Monument) to minimize potential for accidental or unintentional contamination of populations of other subspecies. The habitat must be spatially separated from Quitobaquito Spring such that any natural or human-induced catastrophe would be unlikely to impact both populations. Transplant stocks must be obtained directly from Quitobaquito Spring and comprised of not fewer than 500 fish with an approximate 1:1 sex ratio. As with transplant populations of the Colorado River form, this refugium population must be self-sustaining within a natural or quasi-natural habitat and capable of persistence without human intervention.

An evaluation of previous transplant success attempts should also be made to guide selection of the refugium site.

TASK 4. DEVELOP PROTOCOLS FOR EXCHANGE OF GENETIC MATERIAL AMONG DESERT PUPFISH POPULATIONS

Recent research has demonstrated that several refugium populations of desert pupfish differ little from their parental natural populations (Turner 1984), suggesting that transplanted populations can be a biologically valid component of management and conservation. However, other studies with captive populations of closely related species indicated there is loss of some rare alleles found in natural populations (Edds and Echelle 1989). This indicates that maintaining the genetic integrity of transplanted populations requires adherence to specific management recommendations (see also Echelle 1988 and 1991).

Initial studies by Turner (1983) compared samples from pupfish populations at six localities and detected allozyme differences among stocks from Salton Sea, Cienega de Santa Clara, and Quitobaquito Spring. The overall level of differentiation was low and in the range of within-population comparisons in other teleosts. These data must be expanded 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 must 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 re-established populations. Applicable recommendations to establish such a protocol have been suggested (Echelle 1988 and 1991, Edds and Echelle 1989 and references therein).

Development of this protocol will involve using quantitative modelling techniques to determine the frequency and number of individuals to be exchanged between populations and to ensure that each desert pupfish stock maintains its genetic integrity. This integrity should be maintained so the populations' genetic diversity is allowed to follow a natural, independent evolutionary path. Some genetic changes may have already occurred in desert pupfish as a result of human induced or other factors.

TASK 5. MONITOR AND MAINTAIN NATURAL, RE-ESTABLISHED, AND REFUGIUM POPULATIONS

Two levels of population monitoring are necessary to assess population status, detect trends, and evaluate success of desert pupfish recovery. The first is twice-annual assessment of population and habitat condition, and the second is periodic (5-year interval) examination of population genetics. Monitoring schedules may be modified after populations have established and their security is assured.

Population monitoring should be conducted before spawning commences in spring and again in late summer-early autumn. All populations, natural, re-established, and refugium, must be examined. The spring sampling would provide an index of adult abundance after over-winter mortality, and the late summer-autumn sampling would allow assessment of reproductive success and probable recruitment. As practicable, all populations should be monitored within the same general timeframe so that seasonal effects on population dynamics do not confound interpretation of data. Qualitative estimates of adult numbers may be accomplished by either surface or underwater inspection. Where circumstances warrant (e.g., spatially large or complex habitats where competent visual estimates of population size are not possible) population estimates by quantitative methods such as mark-recapture may be necessary. Monitoring protocols should be standardized (e.g., methods, equipment, length of sampling, number of observers, etc.) within, and to the extent practicable, among sites.

Habitat assessments and population estimates should be conducted coincidentally, under site-specific protocols mutually established by the Service and other responsible management agencies. Methods must be sufficient to detect changes in habitat quality and the status of native and non-native fishes. Requisite data may vary among locales but will include location, technique, temperature, water depth, clarity, flow, surface area, diversity and abundance of aquatic vascular plants and algae, weather, and condition of banks, substrate, and riparian areas. Representative habitat conditions at each site should be photo-documented at fixed locations. Changes in habitat other than those reliably ascribed to seasonal variation must be assessed for potential impact to resident pupfish. Data acquired during routine monitoring will be integrated with studies to determine factors affecting persistence of desert pupfish populations (Task 6). All data collected during population and habitat monitoring will be submitted to a Service designated, central repository/clearing house for distribution and permanent archiving.

Genetic monitoring of populations should be accomplished at 5-year intervals using fish collected during population/habitat assessments. Screening of the appropriate number of diagnostic loci should be performed to determine the rate and nature of change in genetic composition, if any, and to provide additional modelling data as necessary. Samples of approximately 50 pupfish (25 males and 25 females) should be collected from each population, fully and accurately labeled, fresh-frozen, and stored in a supercold freezer until analyzed. Substantial short-term changes would not normally be expected to occur within natural populations, and lack of change can be interpreted as indication that populations are genetically stable. Where changes occur, their implications must be expediently and thoroughly assessed by qualified persons so that necessary adjustments to recovery protocols can be planned and implemented. It is anticipated that this recovery plan will undergo revision as new information becomes available.

TASK 6. DETERMINE FACTORS AFFECTING POPULATION PERSISTENCE

Many attempts to prevent the demise and to establish new desert pupfish populations have failed. Although factors such as habitat size and stability, water quality, minimum population size, and non-native species have been suggested as being important influences, there has been little attention given to quantifying causal relationships and designing programs to maintain populations and maximize population establishment success. Success rates may be improved by quantifying habitat and life history characteristics and applying basic principles of conservation biology. With this information, populations may be established and managed by incorporating a thorough understanding of population and genetic demographics and habitat requirements into consideration of requirements to secure populations. The research efforts described in this section are considered valuable adjuncts but secondary in implementation priority to recovery tasks 1-5 above. Information derived from this research is nonetheless expected to prove essential to desert pupfish recovery.

Life history and habitat preference information is required also to establish criteria for selecting refugia on merits of their ability to provide population security. An understanding of life history and habitat preference is required to determine the viability and status of native populations, to develop delisting criteria, and rehabilitate habitats so they may be better suited to desert pupfish than to non-natives.

6.1 Develop Habitat Criteria

The size of desert pupfish populations is influenced by habitat size and quality. Habitat preference and additional physico-chemical tolerance information is required to determine size and quality of habitat necessary to support secure populations, both in natural and re-establishment sites.

Habitat parameters that may be important include water depth, water quality and quantity, annual temperature regime, substrate, cover, aquatic vegetation, and current velocity. These studies need to examine requirements for reproduction, juvenile rearing, and feeding. Habitat preferenda of common non-native species occurring in desert pupfish habitats must also be determined. This may in the future make it possible to create habitat suitable for pupfish but poorly suited to occupation by introduced species. Being able to manage habitats in this manner should decrease the incidence of non-native species becoming established in desert pupfish habitats.

6.2 Determine Biological Criteria

The influences that habitat quality and biological factors have on population size and persistence are difficult to segregate because population viability is a function of interactions between abiotic and biotic factors. It is important that such factors be examined to identify tasks for quantification of minimum viable population size, description of a biologically secure population, and preparation of delisting criteria.

Control of non-native aquatic species is a primary requirement for recovery of the desert pupfish throughout its range. This control will be difficult because non-native species are widespread and persist in a wide variety of environments; they will be difficult to eliminate from desert pupfish habitats. Quantification of the effects of these species on desert pupfish will provide information that will assist in managing native and refugium habitats so the influence of these species on desert pupfish is minimized or eliminated.

In order to determine the effects of non-native species on desert pupfish, it is necessary to understand the life history and habitat requirements of all species in the assemblage. Once understood, it will be possible to determine areas of niche overlap and segregation and identify which non-native species impact desert pupfish. Integration of these data and knowledge of habitat preferenda for desert pupfish will permit implementation of management actions to enhance pupfish but discourage or eliminate non-native species.

6.3 Acquire Desert Pupfish Life History Information

Detailed life history information is required to determine characteristics of desert pupfish population dynamics. It is important that parameters such as the mean and variance of population increase, effective population size etc., required to develop life tables be determined. These studies must also evaluate the effects of demographic, genetic, environmental, and catastrophic events to determine the probability of extinction within, for example, the next century and millennium. This will permit quantification of requirements to maintain viable populations in small habitats that may be influenced by factors such as catastrophic events and introductions of non-native species.

TASK 7. INFORMATION AND EDUCATION

An information and education program is needed to inform the public, resource managers, and others of the desert pupfish and its plight. This program could include videotape and slide presentations, brochures and pamphlets, seminars, training sessions, and other information-exchange meetings; these should be available in both English and Spanish.

The purpose of education is two-fold. First, it provides an opportunity for the general public to become aware of and informed about, the pupfish and its plight, and about the ecosystem-level implications of species extinction. Strong support for rare species conservation can be derived from a knowledgeable public. For example, a multi-media campaign launched in behalf of the Devils Hole pupfish (*Cyprinodon diabolis*) not only benefitted this

imperiled species but also had profound influence on passage of the Endangered Species Act (Deacon and Williams 1991). A public constituency who understands and appreciates that perpetuation of endangered species requires protection of environments upon which the species depend for survival, and upon which people ultimately depend, is an invaluable ally for recovery.

Second, there are individuals within the resource management community who require training in endangered species conservation and in their legal obligations under the Endangered Species Act. These individuals may represent any level of several involved State or Federal agencies, plus the academic and private sectors. Needs of these individuals should be addressed through workshops, training seminars, and participation in public information and education programs.

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Glossary of Terminology

CAPTIVE -- populations outside of historic range and/or in aquaria, pools, ponds or chambers, where water must be supplied to historically unwatered habitats.

CIENEGA -- mid-elevation (1,000-2,000 m) wetlands characterized by permanently saturated, highly organic, reducing soils, and a depauperate flora dominated by low sedges highly adapted to such soils (Hendrickson and Minckley 1985).

EJIDO -- communal farm.

LENTIC -- relating to still waters, as in ponds.

NATIVE -- a species within its historic range.

NATURAL -- relatively free of human or human-induced impact; in a condition approximating that which existed prior to manipulation by technologic humans.

NATURAL POPULATIONS -- those remaining populations occupying historic habitats and which were not known to have been placed in those habitats by humans.

NON-NATIVE (EXOTIC) -- species introduced outside their native range.

PANMIXIA -- random mating within a breeding population.

QUASI-NATURAL -- constructed or modified for the specific purpose of imitating a natural habitat.

RE-ESTABLISHED -- reintroduced populations, within historic range, where documentation of earlier presence at that specific site may not exist.

SECURE -- protected from human or human-induced impacts; further defined for desert pupfish as formal protection of habitat and water rights by methods such as land and water rights acquisition, legislation, or management agreement, and maintenance of a genetically pure viable population.

TELEOSTS -- any group of fishes with a bony rather than a cartilaginous skeleton.

VIABLE POPULATION -- capable of maintaining itself over the long term without human manipulation; in the case of desert pupfish, until additional information becomes available a viable population will include not fewer than 500 overwintering adults, or existing numbers, whichever is greater, in a normal sex ratio and with in-situ reproduction and recruitment sufficient to maintain that number.

III. IMPLEMENTATION SCHEDULE

PRI-ORITY #	TASK #	TASK DESCRIPTION	TASK DURA-TION (YRS)	RESPONSIBLE PARTY: SERVICE: REG.	RESPONSIBLE PARTY: SERVICE: PROG.	RESPONSIBLE PARTY: OTHER	COST ESTIMATES ($000'S): FY 1994	FY 1995	FY 1996	FY 1997	FY 1998	COMMENTS
1	1.1	Identify ownership of natural habitats	1	2*,1	ES,RE		1,1	0	0	0	0	
						AGFD	1	0	0	0	0	
						CADFG	1	0	0	0	0	
						CES	1	0	0	0	0	
						BLM	1	0	0	0	0	
1	1.2	Acquire natural habitats	15	2*,1	ES,RE,RW		1,2,2	1,2,2	1,2,2	1,2,2	0,1,1	
						AGFD	0	5	5	5	3	
						CADFG	5	5	5	5	0	Not including acquisition costs
						CES	+	+	+	+	+	
						BLM	5	10	5	5	5	
						~~TNC~~	~~0~~	~~0~~	~~5~~	0	0	
1	1.3	Secure natural populations and habitats	15	2*,1	ES,RW,LE		~~1,0,0~~	~~2,2,1~~	~~2,2,1~~	~~1~~	1	
						AGFD	~~1~~	~~5~~	~~5~~	~~5~~	5	
						CADFG	~~1~~	~~5~~	~~5~~	~~5~~	5	
						CES	~~+~~	~~+~~	~~+~~	~~+~~	+	
						BLM	~~1~~	~~5~~	~~5~~	~~0~~	5	
						NPS	~~1~~	~~5~~	~~0~~	~~5~~	0	
1	4.0	Genetic exchange protocol	5	2*,1	ES		10	10	10	10	10	
						AGFD	5	5	5	5	5	
						CADFG	5	5	5	5	5	
						CES	+	+	+	+	+	
1	5.0	Monitor and maintain populations	on-going	2*,1	ES		8	23	8	10	10	
						AGFD						
						CADFG	**	**	**	**	**	
						CES						
						BLM						
						NPS						
						FS						
2	2.0	Re-establish populations	15	2*,1	ES,FR		20	20	20	20	20	
						AGFD						
						CADFG	**	**	**	**	**	
						CES						
						BLM						
						TNC						
						NPS						
						FS						

* - Lead region
** - Due to undetermined ownership of potential re-establishment sites, costs cannot be assigned.
\+ - Due to economic differences and administrative re-configuration of respective Mexican agencies, we are unable to provide estimates for Mexico's responsibility.

IMPLEMENTATION SCHEDULE

PRI-ORITY #	TASK #	TASK DESCRIPTION	TASK DURA-TION (YRS)	RESPONSIBLE PARTY: SERVICE REG.	RESPONSIBLE PARTY: SERVICE PROG.	RESPONSIBLE PARTY: OTHER	COST ESTIMATES ($000'S) FY 1993	FY 1994	FY 1995	FY 1996	FY 1997	COMMENTS
2	3.0	Establish Quitobaquito refugia	15	2*	ES		2	2	1	1	1	
						AGFD	2	2	1	1	1	
						NPS	6	6	3	3	3	
2	6.1	Develop habitat criteria	5	2*,1	ES		5	5	5	5	1	
						AGFD	0	4	4	4	0	
							0	4	4	4	0	
						CADFG	+	+	+	+	+	
						CES	0	4	4	4	0	
						BLM	0	1	2	2	0	
						FS						
2	6.2	Determine biological criteria	5	2*,1	ES		5	5	5	5	5	
						AGFD	5	5	5	5	5	
							5	5	5	5	5	
						CADFG	+	+	+	+	+	
						CES	5	5	5	5	5	
						BLM						
3	6.3	Acquire life history information	15	2*,1	ES		0	2	2	2	2	
						AGFD	2	5	5	5	5	
							2	5	5	5	5	
						CADFG	+	+	+	+	+	
						CES	2	5	5	5	5	
						BLM						
3	7.0	Information and education	on-going	2*,1	ES		0	0	1	1	1	
					PAO		2	1	1	1	1	
						AGFD	1	1	1	1	1	
							1	1	1	1	1	
						CADFG	+	+	+	+	+	
						CES	1	1	1	1	1	
						BLM	1	1	1	1	1	
						FS	1	1	1	1	1	
						NPS						
						TOTAL	122	184	161	154	126	747

* - Lead region
\+ - Due to economic differences and administrative re-configuration of respective Mexican agencies, we are unable to provide estimates for Mexico's responsibility.

Definitions and Acronyms

Priorities in column one of the following implementation schedule are assigned as follows:

1. Priority 1 - An action that must be taken to prevent extinction or to prevent the species from declining irreversibly in the foreseeable future.

2. **Priority 2** - An action that must be taken to prevent a significant decline in species population/habitat quality or some other significant negative impact short of extinction.

3. **Priority 3** - All other actions necessary to meet the recovery objective.

Key to Acronyms used in Implementation Schedule

AGFD - Arizona Game and Fish Department
BLM - Bureau of Land Management
CADFG - California Department of Fish and Game
CES - Centro Ecológico de Sonora
FS - Forest Service
NPS - National Park Service
TNC - The Nature Conservancy

FR - Fisheries Resources
ES - Ecological Services
LE - Law Enforcement
PAO - Public Affairs Office
RE - Realty
RW - Refuges and Wildlife