Ash Meadows Amargosa Pupfish
(Cyprinodon nevadensis mionectes)

5-Year Review:
Summary and Evaluation

[*Photo by US Fish and Wildlife Service*]

U.S. Fish and Wildlife Service
Nevada Fish and Wildlife Office
Las Vegas, Nevada

Date Signed

March 17, 2010

5-YEAR REVIEW

Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*)

I. GENERAL INFORMATION

Purpose of 5-Year Reviews:

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

Species Overview:

The Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*) is a small killifish (Order Cyprinodontiformes) endemic to the springs within Ash Meadows, Amargosa Valley, Nye County, Nevada (Figure 1). It is a subspecies of a larger group of fish, *C. nevadensis*, endemic to the Amargosa River system, and has likely been isolated as a subspecies since the Pleistocene epoch (~1.8 million to 10,000 years before present (bp)). It occurs in thermal springs, and their outflows, that have a wide variety of physical characteristics. Since the settlement of Euro-Americans within the Amargosa Valley, this subspecies has been subject to a variety of threats, many which have caused a deterioration of habitat. The listing of this subspecies was in response to these threats, and a recovery plan was developed.

Methodology Used to Complete This Review:

This review includes an analysis of life history, research, and survey data available in the U.S. Fish and Wildlife Service (Service), Nevada Fish and Wildlife Office, and Ash Meadows National Wildlife Refuge (Refuge) files; U.S. Geological Survey – Biological Resources Discipline (USGS-BRD) files; Nevada Department of Wildlife files; other data available in general scientific literature and personal communications; and public comment, if received. This review contains updated information on the species' biology and threats, and an assessment of that information compared to that known at the time of listing or since the last 5-year review. The Service focuses on current threats to the species that are attributable to the Act's five listing factors. The review synthesizes all this information to evaluate the listing status of the species and provide an indication of its progress towards recovery. Based on this synthesis and the threats identified in the five-factor analysis, the Service recommends a prioritized list of conservation actions to be completed or initiated within the next 5 years.

Contact Information:

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Federal Register (FR) Notice Citation Announcing Initiation of This Review: A notice announcing initiation of the 5-year review of this taxon and the opening of a 60-day period to receive information from the public was published in the Federal Register on July 7, 2005, 70 FR 39327. A second notice announcing the 5-year review and extending the request for information until January 3, 2006, was published on November 3, 2005 (70 FR 66842). One letter of comment was received from the Center For Biological Diversity.

Listing History:

Original Listing
Emergency Rule
FR Notice: 48 FR 608
Date of Emergency Listing Rule: January 5, 1983
FR Notice: 48 FR 40178
Date of Final Listing Rule: September 2, 1983
Entity Listed: Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*)
Classification: Endangered

State Listing
The Ash Meadows Amargosa Pupfish (*Cyprinodon nevadensis mionectes*) was listed by the State of Nevada as threatened on December 1, 1982, pursuant to Nevada Administrative Code 503.065.

Associated Rulemakings:

Critical habitat was designated at the time of original listings on January 5, 1983 (Emergency Rule) and September 2, 1983 (Final Rule) (48 FR 608-625; 48 FR 40178-40186).

Review History:

The status of the Ash Meadows Amargosa pupfish has not been reviewed since the species was listed in 1983.

Species' Recovery Priority Number at Start of 5-Year Review: 15

Reported in the 2009 annual recovery data call. A priority number of 15 reflects a low degree of threat with a high potential for recovery as applied to a subspecies.

Recovery Plan or Outline

Name of Plan or Outline:

Recovery Plan for the Endangered and Threatened Species of Ash Meadows, Nevada

Date Issued:

September 28, 1990

II. REVIEW ANALYSIS

Application of the 1996 Distinct Population Segment (DPS) Policy

The Endangered Species Act defines "species" as including any subspecies of fish or wildlife or plants, and any distinct population segment (DPS) of any species of vertebrate wildlife. This definition of species under the Act limits listing as distinct population segments to species of vertebrate fish or wildlife. The 1996 Policy Regarding the Recognition of Distinct Vertebrate Population Segments under the Endangered Species act (61 FR 4722, February 7, 1996) clarifies the interpretation of the phrase "distinct population segment" for the purposes of listing, delisting, and reclassifying species under the Act. This subspecies of pupfish occurs in a limited area and no DPS has been established.

Information on the Species and its Status

Species Biology and Life History

The Amargosa pupfish (*C. nevadensis*) (Eigenmann and Eigenmann), described in 1889, is of the Class Actinopterygii, Order Cyprinodontoformes, Family Cyprinodontidae, and subfamily Cyprinodontinae. La Rivers (1994) and Miller (1948) described the taxonomy of this species, which contained six subspecies: *calidae, amargosae, nevadensis, shoshone, mionectes*, and *pectoralis*. The holotype and paratypes for *mionectes* were collected from Big Springs in Ash Meadows in 1942 by Miller, and was named in 1948.

Based on molecular clock techniques, Echelle, et al. (2005) suggested the genus *Cyprinodon* diverged from the genus *Megupsilon* as late as the Late Miocene (7-9 million years bp), which correlates with information obtained examining the genetic information and fossil record of the conspecific Goodeidae and Hydrobiid snails (Hurt 2004). Approximately 3.6 to 3.8 million years bp pupfish occurred in the Death Valley/Amargosa River paleosystem (Echelle, et al 2005). The only known fossil of a *Cyprinodon* is from Death Valley, and dated to the Late Miocene to early Pliocene (Miller 1981). It is likely that the current *C. nevadensis* complex is the result of two separate invasions of ancestral pupfish to the Death Valley/Amargosa River paleosystem (Echelle and Dowling 1992), which along with the geologic evolution of paleorivers, could explain the discrepancies within the time periods. *C. n. mionectes* developed into its current form following the Pleistocene ice age (approximately 10-20 thousand years ago), as the climate dried and populations became isolated (Duvernell and Turner 1998). *Cyprinodon nevadensis* is the most morphologically variable species of pupfish, but can be defined by the following combination of characters: scale surface deeply reticulate, circuli without obvious spine-like projections; scales large, usually 25 to 26 in lateral series; central cusp of tricuspid teeth narrower than outer cusps; and breeding color of males deep blue and without yellow color (Miller 1943; Moyle 1976). The subspecies *C. n. mionectes* differs by a scale and finray counts less than average for the species; reduced body size; short, deep, and slab-sided body with a greatly arched and compressed predorsal profile; and a very long head and opercle. Of the lesser scale counts, the number around the caudal peduncle was particularly diagnostic (mean = 13 – 15 scales vs. 15.4 – 16 scales for other subspecies). Generally, the pupfish is less than 2 inches (50 millimeters) in length. Variation also exists among the various populations of *C. n. mionectes* (Miller 1948). Lema and Nevitt (2006 and references therein; also Watters, et al. 2003) demonstrate that there is phenotypic plasticity in *C. nevadensis*, which was influenced by several environmental characteristics that in part translated to growth and developmental characteristics. Examples of this included morphometric and other physical differences when comparing individuals having differing growth rates (i.e. enlarged heads and reduced pelvic fins). Based on this information, we maintain that *C. n. mionectes* is a valid taxon without varietal distinctions and is currently restricted to Ash Meadows. This determination is consistent with the conclusions of the 1983 listing action.

Minimal life history information has been gathered for Ash Meadows Amargosa pupfish; however, a significant body of literature exists on *Cyprinodon* physical tolerances in general given the harshness of the habitats in which they live. In addition, some genetic work has been completed on this genus to understand habitat tolerance, principles of speciation due to biogeography, and to ascertain pathways of aquatic species movement over geologic time. Given the nature of the existing body of literature, generalizations must be used when comparing *C. n. mionectes* to other subspecies or closely related species of pupfish. Pupfish are relatively short-lived species, with a life span of two to four years (Scoppettone et al. 1995). Soltz and Naiman (1978) provide a summary of life history and growth traits of pupfish, including *C. n. mionectes*, which is summarized below. Longevity is related to water temperature, and is a function of metabolism. Typically pupfish living in warm waters reach maturity at two to four months, and then live six to nine months as an adult. In colder waters, such as spring outflow tailwaters or marshes, pupfish may go dormant during winter, ultimately extending their lives to approximately three years. As with most fish species, pupfish in harsher environments have more drastic survivorship curves for juveniles than fishes in stable environments. The highest death rate occurs during juvenile and early adolescent life stages in unstable, harsh environments, as opposed to juveniles and adults in a stable environment. Pupfish mature very quickly, and grow approximately 9 percent of their body mass per day as opposed to 1 percent for adults, depending upon available resources and physical habitat. Growth is highly dependant on environmental temperature, and fish in constant warm water (typically spring-dependant temperatures) grow year-round whereas fish in variable cooler waters (typically atmospheric-dependant temperatures) grow at lower rates.

One of the unique traits of *Cyprinodon nevadensis* is that they are highly eurythermal, and several investigations regarding their thermal tolerance have been completed (McCauley and Thomson 1988, Brown 1971, Otto and Gerking 1973). Pupfish tolerate a wide variety of temperatures ranging from 35.6 to 111.2 degrees Fahrenheit (2 to 44 degrees Celsius) (Feldmeth 1981). In the laboratory, Hirshfield et al. (1980) determined *C. n. mionectes* taken from the Big Springs pool and acclimatized to standard temperature and oxygen levels to have a thermal minimum of approximately 36.8 degrees Fahrenheit and a maximum of approximately 107.0 degrees Fahrenheit (2.7 to 41.7 degrees Celsius), which was significantly less variable than the Amargosa pupfish (*C. n. amargosae*), which is adapted to a more variable habitat. These thermal limits are the extremes for survival that were developed in a closed tank, and tolerances for oogenesis and egg development are much narrower (Shrode and Gerking 1977). In addition, activities such as feeding or breeding would likely not occur at the extreme temperatures. Hirshfield et al. (1980) report critical oxygen minima to be 1.66 parts per million; however, these fish were also acclimatized, originated from the stable Big Spring pool, and the minima is likely to be higher relevant to development and other activities such as sustained feeding or breeding.

Most of the spring systems within the Mojave Desert are alkaline, and pupfish are susceptible to low pHs. Lee and Gerking (1980) determined critical minima and effects of low pH on *C. n. nevadensis*. A 96-hour LC_{50} test determined 50 percent mortality at the pH of 4.56 after 96 hours. Egg production, as well as reduced number and development of oocytes in the ovaries, were curtailed in all situations more acidic than a pH of 8.3, and egg production essentially ceased at below 5.0. Egg viability was reduced 50 percent as pH dropped from 8.3 to 6.5. Lee and Gerking (1980) also found that larvae were less tolerant to pH stress than were adults.

Pupfish in general have a very wide tolerance to salinity, and pupfish from within the Colorado River/Death Valley system have been maintained and reproduced in water ranging from distilled water to a salinity 2.5 times saltier than seawater (with some fish surviving in water up to 3.7 times saltier). This is due to their unique ability to rapidly adjust serum osmotic concentration of ions, preventing water loss (Soltz and Naiman 1978).

Food

All pupfish have similar diets, essentially being omnivores and detritivores (Soltz and Naiman, 1978). The primary food for the pupfish is periphyton and algae, but they also consume invertebrates, detritus, and diatoms (Moyle 1976; Naiman 1979; Scoppettone et al. 1995). It is likely that most Mojave Desert fishes within the Cyprinodontidae and Goodeidae have similar diets, as they fill similar niches and have similar morphology. One exception may be the formerly co-occurring *C. n. mionectes* and *Empetrichthys merriami*, (the latter is the now extinct Ash Meadows killifish), which likely had a niche breadth separation. Soltz and Naiman (1978) suggested there was intense resource competition between these two species and therefore the killifish occupied only the deeper pool habitats. In addition, there are several slight differences, such as *E. merriami* having a slightly more carnivorous diet (Soltz and Naiman 1978).

Spawning

Spawning peaks in the spring, but occurs from April to October, and the size of each population fluctuates throughout the course of a year (Soltz and Naiman 1978). Although significantly regulated by diel light cycles and partially by water temperature, spawning likely occurs year-round, especially in warmer habitat. Pupfish reproduce in waters of 77-88 degrees Fahrenheit (25-31 degrees Celsius) (Gerkin and Lee 1983). The individuals in the springs and stable habitats likely have a different reproductive strategy than at the spring outflows with harsher, variable conditions or in ephemeral habitats, where population numbers likely fluctuate greatly depending on conditions.

Spatial Distribution

Distribution of this species includes nearly all of the surface waters within the Refuge, inholdings, and adjacent land in the Amargosa Valley, Nye County, Nevada (Figure 1), with the exception of Devils Hole, the Warm Springs Complex, Crystal Reservoir, reservoirs or other sites with poor water quality, and isolated springs and seeps with no historic connection or which are seasonal.

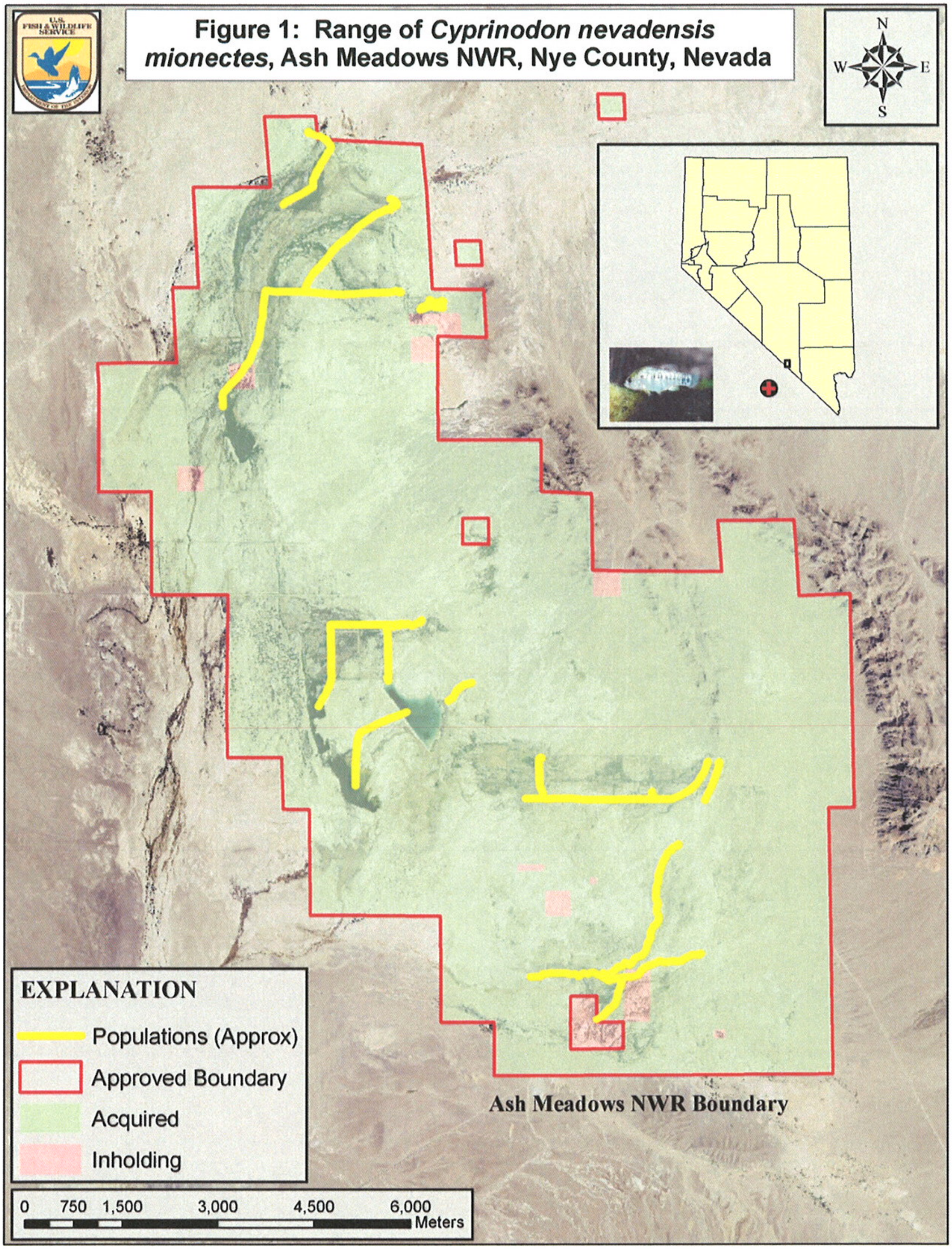

Figure 1: Distribution of the Ash Meadows Amargosa pupfish prepared for 2008 5-year review.

Abundance

The Ash Meadows Amargosa pupfish is a common fish relative to other endemic fishes on the Refuge (Scoppettone et al. 1995), being fairly widespread in suitable springs and their outflows. Abundance of pupfish varies depending on habitat. Pupfish in lotic habitats, as opposed to lentic (predominantly spring pool) fish, are highly variable in population size, changing 10 to 20 times in magnitude over the course of a year. Abundance may also be affected by behavior (Soltz and Naiman 1978). Pupfish have been shown to change habitat use depending on time-of-day, and may migrate to cooler waters during the hotter portions of the day. However, Scoppettone et al. (1995) suggests that the pupfish at Ash Meadows do not appear to migrate as such. This behavior may be localized at extreme conditions at sites such as Tecopa, where pupfish are restricted to sheet flows and stream pools.

Quantitative surveys of specific sites are conducted on at least a biennial basis, usually with mark-recapture surveys and using the basic Petersen equation ($N=M*C/R$)[1] to estimate abundance. Ninety-five percent Confidence Intervals were determined based on the Poisson distribution (Ricker 1975). Additional surveys included snorkel surveys and total removal prior to management efforts, such as draining ditches. All known quantitative survey estimates are reported in Table 1 (pupfish numbers).

	1989	*1990*	*1991*	*1992*	*1993*	*Spring 1994*	*Fall 1994*	*1995*	*1996*	*1997*	*1998*	*1999*	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*
Fairbanks Spring	912				801		677	1147		1799		2800	2348		1146		2262		608
Rogers Spring	114				102		171	124		332	350	416	406		219		188		550
Longstreet Spring	866	548	610	781	636		396	552		990	1386	1327	1210		799		1185		466
Five Springs										75		250							
Crystal Spring	4477	3433	2157	2008	743	384	2909		1556		2890	3046		1156				4288	
Crystal ditch						112			5315^										
Big Spring	1596				1569		1702	2229		1998		1330		2741					
Jackrabbit Spring	406	546	571		820		645		1091		1490	1482		614				1027	
Point of Rocks Spring	>5000								2556!									437	237
Kings Pool	1124				420	527	818	922		1086	1717	1812	1480					1208	
Kings Stream											2167*								
Bradford Spring	1	0					0				0	0							
Forrest Spring	250					0	0												
Tubbs Spring					0	0	0		0										
Clay Pits Spring	>500																		
Crystal Reservoir	>5000																		
Peterson Reservoir	>10000																		

! = Fish in pond before restoration
* = Numbers etimated from snorkel survey
^ = Fish removed from channel prior to drainage

Table 1: Summary of Population Estimates of Ash Meadows Amargosa Pupfish.

Despite relatively low R^2 values and moderately variable confidence intervals, the data suggest that overall trends for the species are stable to slightly decreasing (Figure 2). One noticeable exception is the trend for Crystal Spring and outflow, which appears to be increasing. The trend line on Figure 2 accounts for 70 percent of the variability. These surveys were conducted primarily in the spring pools; however, Soltz and Naiman (1978) indicate that most pupfish occur downstream in the outflow and marsh habitat. Observations throughout the Refuge suggest that in fact *C. nevadensis* ssp. are frequently very abundant in outflows and flooded sites (Scoppettone et al. 1995), which cannot be effectively censused using conventional methods. It is likely that these existing data are useful as indices of population trend since mark-recapture census methods have remained the same, especially relevant to isolated populations separated by barriers, or when sampled from contiguous outflows such as at Crystal Spring. Due to the variable nature of populations in outflows, attempts to characterize data should be used with caution. Additional information regarding Refuge-wide abundance is being collected by the USGS.

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[1] N = number of fish; M = number of fish marked first event; C = number of fish captured second event; R = number of marked fish captured during second event.

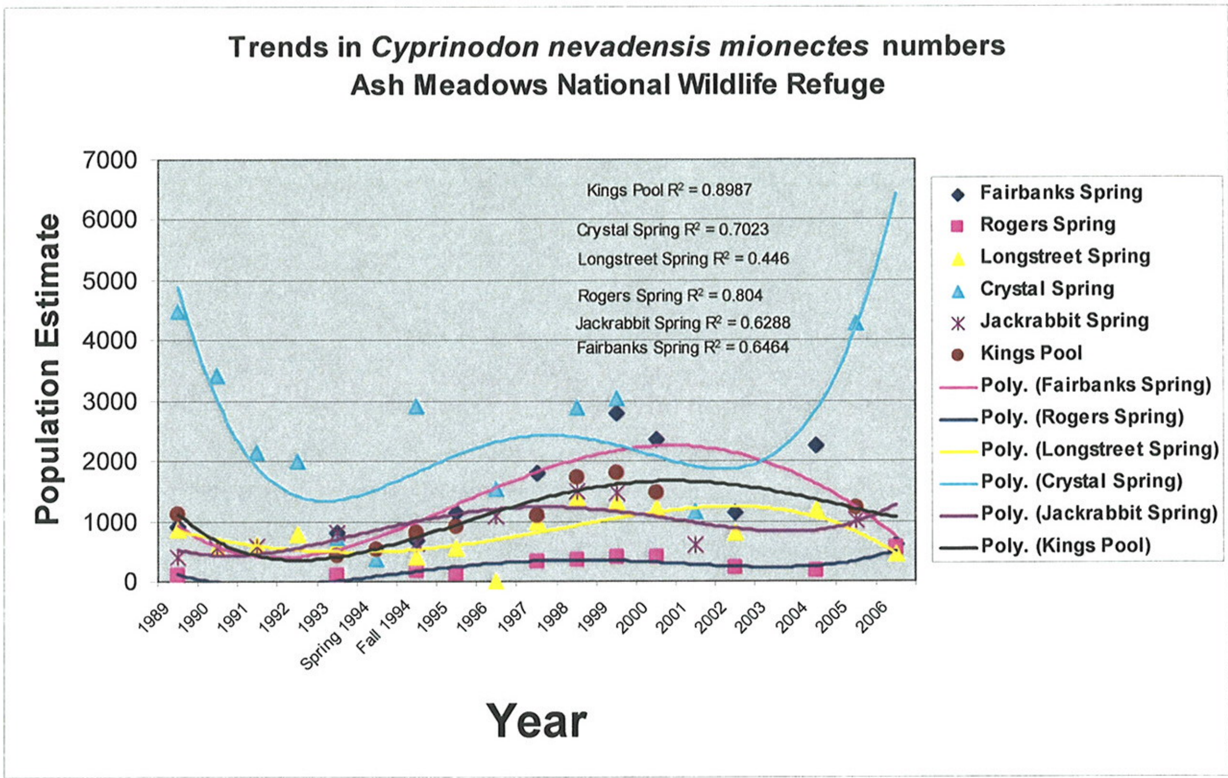

Figure 2: Ash Meadows Amargosa pupfish population numbers within major springs and polynomial (4th order) regression trend lines prepared for 2008 5-year review.

Habitat or Ecosystem

Habitat of this pupfish includes most of the surface waters in the area, virtually all of which were altered during agricultural development prior to Refuge establishment. Pupfish are found in both lentic and lotic habitats. Lotic habitats appear to harbor greater numbers of pupfish; whereas lentic habitats contain fewer, larger, more colorful fish that are more territorial. This morphological and behavioral difference is likely due to additional energy requirements needed to maintain position in lotic habitats. In general, pupfish occupy waters either higher in velocity, and/or deeper, than non-native fish (Scoppettone et al. 1995). Pupfish also prefer warmer sites; often being absent or in low densities in the cooler springs and outflows.

Most springs were altered in several ways: by diversion into earthen or concrete channels, impoundments, drying due to pumping of local groundwater, and/or elimination of native riparian vegetation. These alterations enhanced habitat for non-native predatory and competing aquatic species, hence these altered springs support the widest variety of introduced organisms (Soltz and Naiman 1978; Williams and Sada 1985). Pupfish have dispersed throughout this modified habitat and occur in many habitat types, including sheet flows or seasonal impoundments that are as shallow as approximately 0.5 inch (13 millimeters) deep and that are relatively free of non-native fish.

Terrestrial habitat, both upland and riparian, is important to the pupfish due to allochthonous inputs. Historically, a diversity of vegetation formed a major part of the energy of the ecosystem by facilitating a varied assortment of allochthonous material, such as insects, leaves, and other organic materials, which supported all the trophic levels in the system. In many places within pupfish habitat, this is being replaced by monocultures of non-native weeds, such as salt cedar

(*Tamarix spp.*) and Russian knapweed (*Acroptilon repens*). This has reduced the variety of allochthonous materials, and has caused some of the habitat to become nutrient poor (Kennedy, et al. 2004). Nutrient poor habitat supports lower numbers of fish.

As discussed, several investigations of tolerance within pupfish populations have elucidated habitat requirements, and it appears pupfish can survive in a wide range of conditions. Hirshfield et al. (1980) suggested that there was a genetic difference in thermal and oxygen tolerance between *C. n. mionectes* and *C. n. amargosae*. Oxygen tolerance varied between the subspecies, as with temperature tolerance, suggesting use of oxygen-poor mud substrates for dormancy, temperature refuge, and hiding from predators (also see Soltz and Naiman 1978). Soltz and Naiman (1978) also suggested pupfish were tolerant of high concentrations of H_2S, which is associated with a decomposing organic substrate.

Changes in Taxonomic Classification or Nomenclature

No changes in taxonomic classification or nomenclature have occurred.

Genetics

There have been several investigations regarding the genetic characteristics of *Cyprinodon nevadensis*, most involving *C. n. mionectes*. Echelle, et al. (2005) analyzed mitochondrial DNA of the genus *Cyprinodon* to investigate the phylogenetic relationships between species, and concluded that species within the Death Valley/Ash Meadows group have a common ancestor(s). Duvernell and Turner (1999) determined the Ash Meadows subspecies of *C. nevadensis* to be genetically distinct as a group from the other subspecies associated with the Amargosa River, and Echelle and Dowling (1992) found *C. n. mionectes* to be divergent in up to three times more mitochondrial DNA sites than all other subspecies. This investigation also determined the large populations contained more diversity in SW1-associated genomic DNA than the smaller populations; therefore, the *C. n. mionectes* that occurred in Big Springs at Ash Meadows was more diverse than the Warm Springs pupfish (*C. n. pectoralis*) in this respect although isolated for the same amount of time. Duvernell and Turner (1998) sampled two populations of *C. n. mionectes* from Ash Meadows, as well as *C. n. pectoralis* and *C. diabolis*, and found *C. n. mionectes* to be polymorphic for two unique mitochondrial DNA haplotypes each. Duvernell and Turner concluded that due to the relatively stable habitat (as opposed to the Amargosa River), pupfish at Ash Meadows likely maintained higher effective population sizes and subsequent genetic diversity. Recently, anthropogenic disturbances have reduced population sizes, causing bottlenecks in genetic diversity.

Species-specific Research and/or Grant-supported Activities

Several genetic markers have been developed by Dr. Andrew Martin of the University of Colorado, to support studies of the pupfish, including the Devils Hole pupfish and the Warm Springs pupfish. Martin and Wilcox (2004) demonstrate that there is limited genetic variation among the springs harboring *C. n. mionectes* than variation that exists for *C. n. pectoralis*. In this study, genetic divergence was best explained by elevation: Higher elevation springs harbored populations with more differences than populations in lower springs that could have been closer or more connected. This study also demonstrated that recent bottlenecks have occurred in some populations of pupfish. Data presented in Martin and Wilcox (2004) also suggest that the population of *C. n. mionectes* in Rogers Spring is more closely related to *C. n.*

pectoralis, than it is to other *C. n. mionectes*; however, additional data need to be collected to investigate and confirm this relationship. This work is currently in progress.
Additional work is underway by Gary Scoppettone (USGS-BRD, Reno, Nevada) to determine an updated distribution, population size, and demographics of this pupfish, as well as, co-occurring fish species. This work is under contract with Ash Meadows National Wildlife Refuge and is anticipated to be completed in 2010.

FIVE-FACTOR ANALYSIS

The following five-factor analysis describes and evaluates the threats attributable to one or more of the five listing factors outlined in section 4(a)(1) of the Act.

FACTOR A: Present or Threatened Destruction, Modification, or Curtailment of Habitat or Range

Groundwater Development

Ash Meadows is situated on the Death Valley groundwater regional flow system, and contacts two hydrologic sub basins: the Ash Meadows groundwater sub basin and the Alkali Flat-Furnace Creek Ranch groundwater sub basin (Figure 3). This regional flow system extends hundreds of miles to the northeast, through geologically complex strata containing a variety of transmissivities, including fractures, and ultimately discharges in portions of southern Nevada and California (Harrill and Bedinger 2005; Laczniak et al. 1999; and citations therein). Characteristics of this region are defined fairly specifically in the USGS groundwater model (MODFLOW-2000) (Belcher, et al. 2006). The boundaries of these sub basins are uncertain, and lateral flow occurs between the two sub basins; therefore, groundwater withdrawal in one may impact the other. Other portions of the flow system downgradient of Ash Meadows may be partially isolated, and dependant on prehistoric basin-fill water (Anderson et al. 2006; Winograd et al. 2005). Given the proximity and predicted flow paths, the springs at Ash Meadows likely receive water from both the Ash Meadows and Alkali Flat-Furnace Creek Ranch sub basins (USGS 2003).

Groundwater development and the threat it posed to the Devils Hole pupfish and its aquatic habitat in Devils Hole was a major factor that precipitated listing of the Ash Meadows endemic species, including the Ash Meadows Amargosa pupfish. At the time the species was proposed for listing in 1983, estimated water pumping at Ash Meadows by Preferred Equities Corporation would have exceeded the annual yield of the aquifer by 225 percent. Four years ago, groundwater pumping in the aquifer reached nearly 200 percent of the annual yield (USGS 2005).

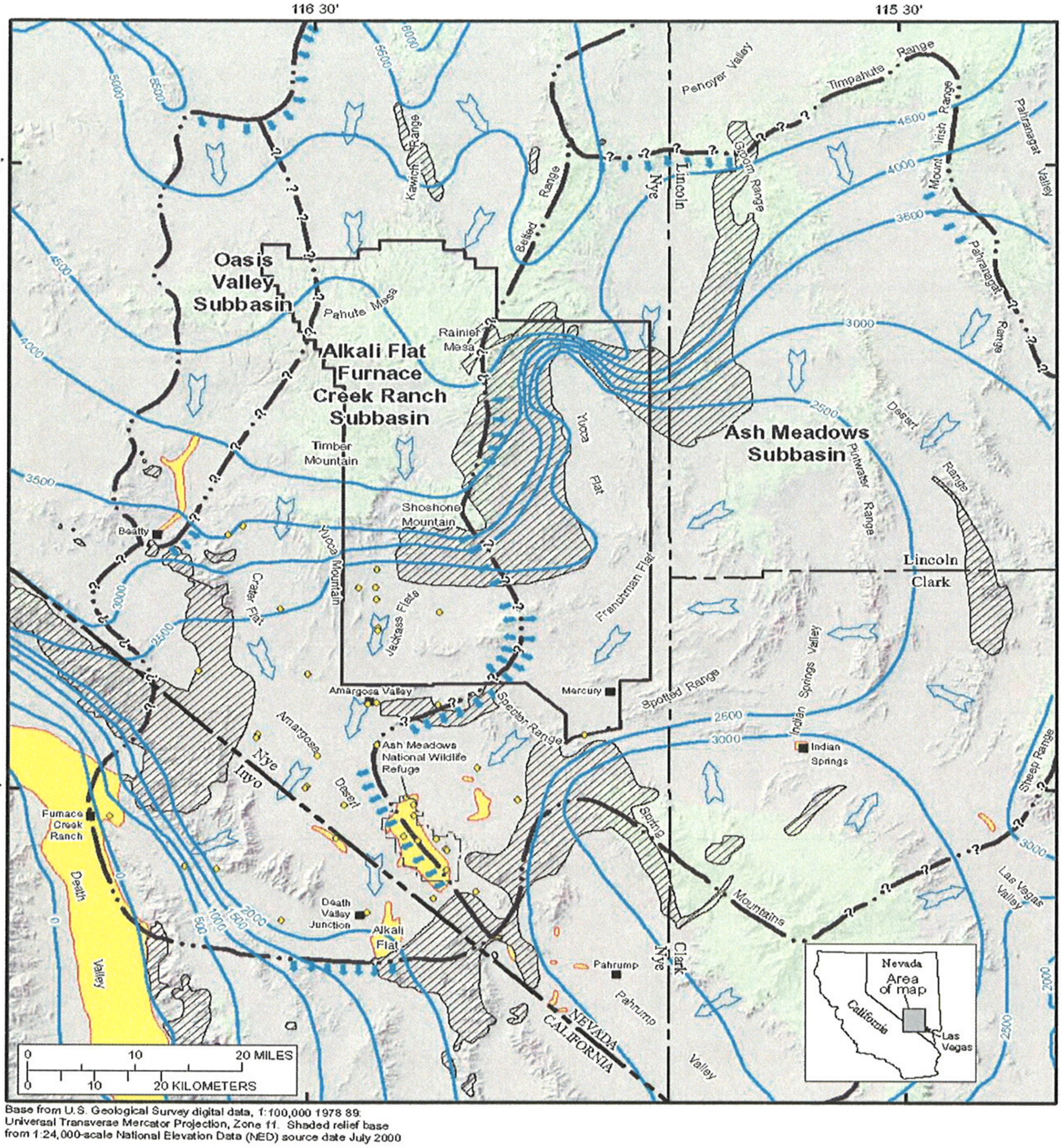

Figure 3: Major Factors Controlling Groundwater Flow in the Yucca Mountain Region, Southern Nevada and Eastern California from 2000 (USGS 2003) prepared for 2009 5-year review.

Spring outflows that comprise pupfish habitat are fully dependant upon groundwater. Groundwater pumping has been shown to affect springs in the Ash Meadows system. Dudley and Larson (1976) described the effects of pumping on springs in Ash Meadows, where several springs demonstrated decreases due to groundwater pumping. Nichols and Akers (1985) described pumping as responsible for an 8.2 meter drop in some Amargosa Valley monitoring wells. Bedinger and Harrill (2006) describe decreasing trends in Devils Hole due to pumping at wells in Ash Meadows, the Amargosa Desert, and the Nevada Test and Training Range.

Groundwater pumping is an incipient threat because the effects to the spring outflows are not likely to be immediately obvious. Effects could be masked by several factors, including annual variations in precipitation, and slow to manifest as the aquifer is heterogeneous and transmissivity varies (Harrill and Bedinger 2005). In addition, there are only limited monitoring efforts[2], and, especially near Ash Meadows, inaccurate information exists regarding the amount of withdrawal among domestic users.

Numerous measures have been implemented that, in part, address this threat. A 1976 Supreme Court decision established a minimum water level in Devils Hole, a 40-ac disjunct unit of Death Valley National Park that occurs within the boundaries of the Refuge, to protect the endangered Devils Hole pupfish (*Cyprinodon diabolis*); as a result of this decision water levels in Devils Hole are carefully monitored. The Service has established water rights for 16,376 acre-feet (ac-ft) (2,020 hectare-meters (ha-m) of annual spring discharge (Mayer 2000, pp. 2-3). This constitutes approximately 96 percent of the 17,025 ac-ft (2, 100 ha-m) annual discharge by the springs and seeps at Ash Meadows (Mayer 2000, pp. 2-3). A groundwater level and spring discharge monitoring program developed by the Service and the USGS in 1998 has been implemented as part of a larger monitoring program for the Amargosa Desert hydrographic basin, which supplies the Ash Meadows region.

On July 16, 2007, the Nevada State Engineer issued Ruling 5750 denying numerous water rights applications in the Amargosa Valley, and finding that the groundwater basin is over-appropriated (State of Nevada 2007, p. 22). On November 4, 2008, the Nevada State Engineer issued Order 1197 further stipulating that any new applications for water rights in the Amargosa Valley will be denied and that changed applications that seek to move pumping more than 0.5 mi (0.8 km) closer to Devils Hole will also be denied (State of Nevada 2008, p. 1). Order 1197, however, provides several exceptions including provisions to allow: 1) a change in the place of diversion of less than 0.5 mi (0.8 km) as long as the place of use remains the same; 2) applications for less than 2.0 ac-ft (0.2 ha-m) per year; and, 3) a process for considering the net impact of changes to multiple existing rights, which could permit changes that are the same or less than the impacts to Devils Hole base rights as long as no new diversions are within 10 mi (16 km) of Devils Hole.

Water levels in Devils Hole stabilized after groundwater pumping on the properties that ultimately became the Refuge stopped in 1975; however, the water level in Devils Hole declined 2.76 inches (7 centimeters) between 1988 and 2004 (NPS 2004). The water level subsequently increased in 2005 following an extremely wet year. Mayer (2006, pp. 19 and 28) indicates

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[2] The Devils Hole aquifer is the subject of ongoing evaluation, including monitoring wells and other geotechnical efforts. These efforts are primarily aimed at determining groundwater patterns relevant to Department of Defense and Department of Energy concerns, as well as determining patterns related to Devils Hole and Death Valley. In addition, there are monitoring efforts to detect effects of groundwater withdrawal to Devils Hole. Few of these efforts have a goal of determining impacts to the spring systems at Ash Meadows, and with any groundwater investigation, more data are needed to comprehend the system.

groundwater monitoring wells and spring discharges on the Refuge are currently stable to slightly declining. After groundwater pumping was ceased on the Refuge, it began to increase in the Amargosa Valley, located about 10 mi (16 km) northwest of the Refuge. In 1987, groundwater pumping in the Amargosa Valley was estimated to be 5,670 ac-ft (699 ha-m) per year (USGS 2005). In 2003, groundwater pumping was estimated to have increased to 13,518 ac-ft (1,667 ha-m) per year (USGS 2005). Most groundwater monitoring wells in the Amargosa Valley have shown a significant decline in water levels since 1992 (USGS 2003), and groundwater pumping is currently occurring in some areas of the basin at about twice the rate predicted to be sustainable (USGS 2005).

Water right acquisition by the Refuge, the 1976 Supreme Court order protecting the water level in Devils Hole, and the recent ruling and order by the Nevada State Engineer have all reduced the imminence and the magnitude of the threat that groundwater pumping poses to the aquatic ecosystems at the Refuge, and species that depend upon them. They have not, however, totally eliminated this threat and the significance of the remaining threat posed by groundwater pumping must be evaluated with respect to each of these measures.

The Supreme Court ruling applies specifically to the water level in the Devils Hole, which is the highest hydrological point within the Refuge. It remains uncertain, however, to what extent maintenance of the court stipulated water level in Devils Hole affords protection to other springfed communities within the Refuge, many of which originate to the north and west of Devils Hole and could potentially be affected by either local groundwater pumping on the few remaining inholdings within the Refuge or by the incremental effects of groundwater pumping in the Amargosa Valley. While the Nevada State Engineer's ruling and order preclude new water right applications within the Amargosa Valley and place constraints on change applications, exceptions are included for applications for less than 2.0 ac-ft (0.2 ha-m) per year and for applications that do not change the place of use. These exceptions, while seemingly minor, could have cumulative effects that result in lowering the groundwater table within the Refuge. While the State Engineer's Order 1197 identifies a process for identifying the net effect of changes to multiple existing rights, the analytical process for evaluating the effects of these changes has not been specified.

Numerous active applications for solar energy projects in the Amargosa Valley north of the Refuge have been received by BLM (BLM 2009). The Service (2008a, pp. 1-3) has expressed concern to BLM over the potential amount of water that would be required, which could be as much as 50,000 ac-ft (6,168 ha-m) per year if projects that use wet-cooled concentrating solar thermal technology or other water-use intensive technologies are approved and implemented. The Amargosa Valley has recently been selected as a Solar Energy Study Area to be fully evaluated for its environmental and resource suitability for large-scale solar energy production (Department of Interior 2009, p. 1). The objective is to provide landscape-scale planning and zoning for solar projects on BLM lands in the West, allowing a more efficient process for permitting and siting responsible solar development. If selected, the Amargosa Valley would be available for projects capable of producing 10 or more megawatts of electricity for distribution to customers through the transmission grid system. Companies that propose projects on that scale in areas already approved for this type of development would be eligible for priority processing.

BLM may also decide to use alternative competitive or non-competitive procedures in processing new solar applications for selected areas.

Since Order 1197 precludes the issuance of new water rights in the basin, developers of these projects must purchase existing rights and file applications to change the manner of use, place of use, and/or the location of pumping. It remains uncertain whether all existing water rights are currently being fully exercised. If they are not, the full utilization of all existing water rights in this over-appropriated basin may lead to a lowering of the groundwater table that affects spring-discharge within the Refuge. Industrial uses may also lead to a reduction in return flows when compared to the current agricultural uses of water in the Amargosa Valley.

Although the Service has established water rights to 96 percent of the spring discharge within the Refuge, the Service will have to demonstrate through analyses that the net impact of any change applications will have a negative effect on Ash Meadows. To the extent that the Service is unsuccessful in demonstrating net negative impacts in at least some of these cases, additional incremental declines in spring discharge may occur at Ash Meadows. Such incremental declines could be difficult to attribute to any particular cause or causes after the fact and, therefore, would be difficult to remedy.

Changes in shallow subsurface water and the basin-fill aquifer may also affect springs. Water levels in shallow wells in the Amargosa Valley show annual fluctuations of more than 3 meters, and daily fluctuations of approximately 0.1 meters due to evapotranspiration (Laczniak et al. 1999). Changes in groundwater flow and elevation could also affect the temperature and chemical content of springs to which pupfish are adapted. The pupfish is dependent on spring systems arising from the carbonate aquifer, as well as its nexus with the basin fill and other local subsurface water. Disruptions to the surface and subsurface hydrology are particularly important threats to the species, and all known populations of the pupfish face this threat. While groundwater protection associated with the Devils Hole pupfish currently appears to be sufficient to maintain appropriate springflow in pupfish habitat; additional monitoring of springflow and groundwater needs to be implemented and contingency plans should be developed.

Because of the uncertainties that exist regarding the potential effects of the full exercise of existing water rights in the Amargosa Valley, the incremental effects of additional pumping or changes in the manner or place of use or location of pumping, and the specifics of the process that the Nevada State Engineer will use to evaluate the net effects of such changes, we are unable to conclude at this time that the threat that groundwater pumping poses to Ash Meadows Amargosa pupfish is no longer significant. Based on this information, we conclude the magnitude of the threat of groundwater development is high and the immediacy to be non-imminent.

Habitat Disturbance

Prior to establishment of the Refuge, disturbance occurred at virtually every location within Ash Meadows. This disturbance was due to domestic use, transportation, mining, farming, ranching, and recreation. Disturbance by early homesteaders and modern development were identified in the listing rule.

Domestic Use (Human History at Ash Meadows – Baseline of Historic Impacts)

Although never heavily populated, there is evidence of human occupation at Ash Meadows dating to 7,000 Years Before Present (BP), suggested by large projectile points relating to the Pinto Period (7,000 to 4,000 BP) (Livingston and Nials 1992). Traditionally, the land that is now the Refuge formed a general boundary area between the Western Shoshone and Southern Paiute.

Both the Paiute and Shoshone utilized the plant resources found at Ash Meadows. Mesquite groves were often claimed and managed by individual families. The Timbisha Shoshone managed mesquite groves for food, and maintained open ponds for hunting waterfowl and to obtain other aquatic food (Fowler et al 2003; Kelly Turner, U.S. Forest Service Cultural Resource Specialist, personal communication, based on communications with Timbisha Shoshone Tribal elders). Pine nuts and game were available in the nearby mountains. The Ash Meadows Paiute practiced horticulture near streams, growing corn, squash, beans, grapes, and sunflowers in the moist soil (McCracken 1992). Within the ethnographically recorded history of the area, Ash Meadows was the location of fall festivals, where extended families would re-unite after their summer foraging cycle and gather with other groups. Native American utilization of the area continued well into the historic period. Pupfish were a resource for Native Americans, which were harvested using baskets and roasted in coals (Kelly Turner, personal communication, based on communications with Timbisha Shoshone Tribal elders). Soltz and Naiman (1978) suggest that the potential for food production, mainly in the sense of fish meal, is enormous due to the high productivity of pupfish. This harvest was likely sustainable and probably occurred mainly in springs where larger pupfish congregated. Springs were also used for other resources, such as cattail harvesting for open water maintenance, materials and food.

Euro-Americans began settling in the area in the 1870s when nearby mining booms attracted ranchers to the native grasses for grazing livestock. However, the alkaline soil in Ash Meadows was not very productive for agriculture and caused the cattle's hooves to deteriorate. Most nineteenth century ranching operations only survived a few years; however, this was when large-scale disturbances to the pupfish's habitat began to occur. During the twentieth century commercial enterprises altered the natural environment at Ash Meadows. Clay deposits were mined and milled at the western edge of the Refuge. The abundant water in Ash Meadows drew the attention of large-scale irrigation agriculture. The modern agricultural enterprises were not successful because of the alkaline soil, which was not realized before large sections of the valley floor were leveled and plowed. Carson Slough was mined for peat in the 1970s, and four large reservoirs (Crystal, Peterson, Horseshoe, and Mud Lake) were created; further altering flow and habitat characteristics for the pupfish in Carson Slough. Because of the quantities of spring water pumped for agriculture, the water levels in Devils Hole dropped, posing a threat to the Devils Hole pupfish. A court order in 1976 halted the pumping, and the Refuge was established nearly a decade later, in 1984.

As Euro-Americans began to inhabit Ash Meadows, a transportation system was developed, culminating in a series of dirt roads. Initially roads were small dirt tracks designed to link springs, habitations, or trade routes, primarily to support the mining industry. These roads became more numerous over time as more were developed for exploratory mining and to service agriculture. These roads affected pupfish in several ways, including altering hydrology, enabling greater access to habitat, fragmenting habitat by creating barriers (e.g. culverts), and degrading water quality through dust, erosion, and chemicals (e.g. oils and antifreeze). Springs that were easily accessible by roads experienced greater impacts from agricultural use, recreation, or vandalism. Upon establishment of the Refuge, road development halted, and restoration efforts are now underway to eliminate unnecessary roads and to alleviate damage caused by roads to the habitats.

Farming at Ash Meadows began with Native American and pioneer use of springs for small fields. Recent history included cotton, forage grass, and alfalfa fields which were also used to graze cattle. Agricultural improvements had serious impacts on pupfish habitat. Pumps were placed over springs, which often completely dried the spring and its outflow. Water diversions and flood irrigation were common, which turned spring systems and ditches into ephemeral streams, stranding and killing pupfish. Pesticides, fertilizers, eroded materials, and nutrients from cattle waste likely degraded water quality, harming pupfish. Cattle were concentrated in riparian areas, which caused damage to both the physical and biological characteristics of the watershed. Water collection dikes were developed surrounding the valley, which eliminated normal floods and inputs of rock substrate and allochthonous materials into the stream systems, preventing regeneration of substrate and flushing flows. Fields were leveled, which changed the hydrology and facilitated the spread of non-native weeds. Altered hydrology of the watershed profoundly affected stream morphology, including channel morphology and physical characteristics of the stream.

Many of the streams at Ash Meadows were channelized, forming ditch-like streams. As the watershed was altered to either make fields or capture water, flushing flows that caused channel definition (sinuosity and dimensions) were eliminated. As a result, all mechanical energy required to shape the channel came from the springflow itself, which typically was not powerful enough to cause sinuosity. Given this situation, there has been substantial downcutting of straight channels which become overgrown by terrestrial vegetation. This leads to very poor biological productivity, and increases damage caused during stochastic events. In addition, flushing flows that typically contributed substrate (woody debris and gravels) were prevented, resulting in relatively simple, highly imbedded substrates which are poor for endemic invertebrate production and overall pupfish productivity. These impacts are somewhat moderated by pupfish being very adaptable to different habitat conditions, and their migration to seasonal habitats that are highly productive. Also, when non-native species are not present, pupfish are able to utilize altered habitats. Creation of these conditions has ceased and Refuge staff are in the early phases of restoring habitat to conditions that existed prior to disturbance by agriculture. Based on this information, we conclude the threat to pupfish habitat is low and the immediacy to be non-imminent.

At the time of listing, destruction of habitat by cattle and wild horses, along with off-highway vehicle (OHV) activity, were considered threats. After establishment of the Refuge, agricultural practices, including livestock grazing were discontinued. Wild horse grazing and OHV activity were stopped or limited by construction of roughly 16 miles (26 km) of fencing on the perimeter of the Refuge in 1995. However, illegal OHV activity has recently become a problem again on the Refuge (Baldino 2006b), and fence repairs and OHV monitoring are ongoing activities at the Refuge. The Refuge added a law enforcement officer to patrol the Refuge in 20XX, which should assist with this issue. Because of the positive management practices on the Refuge, we conclude, destruction of habitat by cattle, wild horses, and illegal OHV activity are no longer significant threats to the pupfish. Based on this information, we conclude the magnitude of the threat of disturbance is low and the immediacy to be non-imminent.

Surface Mining

Mining for clay minerals occurs in the Ash Meadows area. The playa sediments covering much of the Ash Meadows area contain clays and other minerals, which may be considered "uncommon varieties," and therefore could potentially be classified as "locatable minerals" under existing mining laws. Specific specialty clays located in the area include bentonite, sepiolite and saponite; zeolite has also been mined from deposits on lands south of the Refuge and commercial deposits likely occur within the Refuge (Wallace 1999, pp. 15-17). Mineral entry on Federal lands is authorized by the Mining Act of 1872; the program is administered by BLM. Under this program, surface disturbance and impacts to rare species that do not have Federal protection are permissible as long as operations comply with all pertinent Federal and State laws. New mineral claims and subsequent mining could cause direct loss of pupfish habitat, as well as indirect impacts by diverting or draining water away from occupied habitat.

Establishment of the Refuge and BLM Area of Critical Environmental Concern (ACEC) surrounding the Refuge has significantly diminished the threat posed by surface mining. The ACEC is now temporarily closed to new mineral claims while BLM processes an application/petition to withdraw mineral entry. We do not have accurate estimates of either the total acres or population sizes of any Ash Meadows Amargosa pupfish occurrences within the ACEC. Within the Refuge, the Service owns mineral rights on approximately 62 percent of the acres on which the Ash Meadows Amargosa pupfish was known to occur prior to 2008. Mineral entry on these lands is unlikely because obtaining the necessary authorizations would require the project proponent to commit to an extended process that would include a Refuge compatibility analysis. It is unlikely that surface mining would be found compatible with the Refuge's purpose.

Ash Meadows pupfish occur on private lands is in the Five Springs area and on several parcels near the south end of the Refuge. The remaining Ash meadows pupfish habitat is BLM or Refuge land. These lands are open to mineral claims but may be included in lands withdrawals.

A mineral withdrawal will not interfere with valid existing mineral rights. Existing mineral claims for specialty clays exist both within and outside of the Refuge and BLM ACEC (BLM and Service 2000, p. A-6). The significance of the threat posed by mineral entry on BLM or Service lands with public minerals is difficult to assess because there is no available information on the actual occurrence or potential value of minerals on these specific parcels of land. Most of these lands, however, remain open to mineral entry nearly a decade after they were petitioned for withdrawal, so some degree of threat remains.

Given the current surface mining activity described in the vicinity of Ash Meadows and the regulatory temporary withdrawal of mineral entry, we conclude the magnitude of the threat posed by surface mining is moderate and the immediacy is non-imminent.

Invasive non-native species

The listing rule stated that problems presented by non-native species would exacerbate impacts caused by habitat disturbance. Non-native species may adversely affect populations of native species through predation, competition, space, or by being vectors for disease and parasites. Often these influences are synergistic and originate from multiple non-native species occurring in the system. Sailfin molly (*Poecilia latipinna*) and pupfish have been determined not to overlap to the degree that would be detrimental to the pupfish (Scoppettone et al. 1995), but there is still a probability that given a large number of mollies, resources for the pupfish would be diminished. This is also true regarding the effects to pupfish from other non-native aquatic organisms, such as crayfish (*Procambarus clarkii*), turban snail (*Melanoides tuberculata*) and Mosquitofish (*Gambusia affinis*).

Exotic fish may also displace native species, either spatially or behaviorally, to habitats of poorer quality. Crowder and Cooper (1982) noted that predators alter habitat selectivity of prey, causing prey to be selective of more complex habitats. Schlosser (1988) demonstrated that prey selected habitat that decreased probability of predation, which limited the ability of the prey to fully utilize available habitat. This may create avoidance behavior that forces fish into marginal habitats, which would lower fecundity. Pupfish, being territorial and naïve to predators, likely are not displaced. Based on this information, we conclude the threat by competition and displacement is moderate and the immediacy to be imminent.

Disease and predation by aquatic non-native species are discussed below under Factor C. Weeds are analyzed in the discussion regarding Factor E: Other Natural or Manmade Factors Affecting its Continued Existence.

FACTOR B: Overutilization for Commercial, Recreational, Scientific, or Educational Purposes

Over-utilization is not a threat to this species. There are no known instances of the public collecting Ash Meadows Amargosa pupfish, either for commercial or recreational (aquarist) utilization. Scientific and educational utilization are managed under section 10 of the Act and Refuge permitting processes, and occur at a very low, negligible frequency and amount. A full-time Service law enforcement officer is now stationed at Ash Meadows and is focused on detecting and preventing illegal utilization. The presence of Service law enforcement also discourages illegal utilization on adjacent BLM land. Based on this information, we conclude the threat by over-utilization is low and the immediacy to be non-imminent.

FACTOR C: Disease or Predation

One investigation regarding this subspecies occurred in April 2008 by Scott Foott (2008) of the Coleman National Fish Hatchery and Technology Center. This investigation found trematode metacercaria in less than 25 percent of the fish sampled, and pattern of infections suggested impairment of health. Pigments associated with a variety of stressors were present; however, not to a degree that suggested impairment. Bacterial, viral, and other parasitological agent

investigations found only common and normal levels of bacteria, and no parasites were found in blood smears.

It is likely that this species has a natural suite of parasites and diseases to which it is adapted to and cause a low degree of morbidity. Of more concern is disease and parasites potentially introduced through the presence of non-native fish and invertebrates. Many parasites alter behavior (Curio 1988), fitness (Boyce 1979), or escape abilities (Krause and Godin 1994) of their intermediate and definitive hosts. Asian tapeworm (*Bothriocephalus opsarichthydis*) has been found in native fish at other sites in southern Nevada, and likely occurs at Ash Meadows. One likely vector for this species is the sailfin molly. Largemouth bass (*Micropterus salmoides*) in Crystal Reservoir have been determined to be heavily infected by mesenteric nematodes (Goodchild and Weissenfluh *in prep*), which likely use cyclopine copepods as a first intermediate host, and a smaller fish species such as pupfish as a secondary intermediate host. Cyclopine copepods were determined to be very abundant and conspicuous in Crystal Reservoir during fish sampling efforts in 2007 (Goodchild 2007). Another potential vector for parasites is *Melanoides*, which have been found to be an intermediate host for the trematode *Centroces formosanus* in western Utah. In Utah, this trematode infects speckled dace and *Gambusia*. There are no known data on prevalence of nematodes or other parasites in the Ash Meadows Amargosa pupfish. Overall, we conclude the threat to the pupfish from disease is low and non-imminent.

Predation is a significant negative impact to pupfish populations at Ash Meadows. Undisturbed habitat without exotic species likely had a low degree of predation from invertebrates, waterfowl, or cannibalism; or Native Americans. These levels of predation were likely sustainable, may have occurred with elevated numbers of pupfish resulting from habitat management by Native Americans, and were an essential component of the ecosystem. Following major habitat disturbance and introduction of non-native species, predation has much more of a profound effect on pupfish. The Refuge is currently conducting non-native species eradication efforts, and has developed an Integrated Pest Management Plan to guide these efforts.

Two major types of predation occur: egg/larval and juvenile/adult fish. Nearly all non-native aquatic species predate on the eggs or larvae of pupfish. Fish predators, such as young-of-year largemouth bass, green sunfish (*Lepomis cyanellis*), sailfin mollies, and *Gambusia* diminish pupfish recruitment. Although undocumented at Ash Meadows, given the wide range of dietary items suitable to the crayfish, it is likely red swamp crayfish have a major impact on eggs and larval fish with low mobility. Additional studies, such as analyses of stable isotopes, need to be implemented to elucidate this relationship. Stable isotope investigations by Kennedy et al. (2005, 2004) were inconclusive regarding predation by crayfish.

Juvenile and adult fish are commonly predated by non-native centrarchids, bullfrogs (*Rana catesbeiana*), and by crayfish. Ash Meadows Amargosa pupfish have been detected regularly in stomach contents of largemouth bass (Goodchild and Weissenfluh 2007). Pupfish have also been detected in bullfrog stomach contents (J. Eckberg, UNLV, personal communication). While bullfrogs likely have limited effects on the pupfish due to their relatively low numbers and restriction to habitat margins, centrarchids have the ability to totally deplete systems of pupfish. This occurred during 2004 in the Big Spring outflow, where pupfish recolonized from an adjacent marsh only after largemouth bass were removed. (Subsequently bass reinvaded Big Springs.) Predation also preferentially affects pupfish over other non-native species that have a shared evolutionary history with the centrarchids (e.g. Gamradt and Katz 1996; Hobbs and Moody 1998; and Meffe 1984). Typically, when bass and pupfish co-occur at Ash Meadows, pupfish are depleted from the system prior to sailfin mollies and *Gambusia*, which have evolved centrarchid avoidance behavior. When large predators are not present, mollies provide a competitive pressure and may eat pupfish larvae. However, Scoppettone et al. (1995) found that mollies will not replace pupfish populations since there was only a 30-56 percent dietary overlap depending on habitat. Predation is facilitated by non-native habitat, and reservoirs provide ideal habitat for large predatory fish such as sunfish and bass. Currently, Crystal Reservoir contains these species, which have eliminated pupfish from the area. They are also a source population for introductions elsewhere on the Refuge. Based on this information and given the dispersion of pupfish, we conclude the threat is high, and the immediacy of the predation threat is imminent.

FACTOR D: Inadequacy of Existing Regulatory Mechanisms

At the time of Federal listing, this species was not protected by State law, and the proposed and final listing rules that included the Ash Meadows Amargosa pupfish described lack of regulatory mechanisms as a threat. It is now classified as 'Threatened' under Nevada Revised Statute 501.065. In addition, the distribution of the species is almost entirely on Federal lands within the Refuge and on adjacent BLM lands within the Ash Meadows ACEC. The pupfish is now protected by applicable Refuge- and BLM-specific regulations preventing take or disturbance of listed species. The presence of Service law enforcement also discourages illegal utilization on adjacent BLM land. Based on this information, we conclude that this listing factor is no longer a threat.

FACTOR E: Other Natural or Manmade Factors Affecting Its Continued Existence

Aquatic Weeds

Aquatic invasive species are a threat to the Ash Meadows Amargosa pupfish as identified in the original listing rule. While native, the Southern cattail (*Typha domingensis*) is a rapidly colonizing invasive pioneer species. Undisturbed pupfish habitat would have a relatively low cover of cattail, which would be restricted to fringes or terminal marshes, while the majority of the habitat would consist of open water with a caliche/pebble substrate. Altered ecological communities, especially habitat developed through degrading agricultural infrastructure, provide an early successional stage that is invaded by species such as cattail. The cattails then provide structure that slows stream velocity and increases deposition, resulting in a silt/organic substrate. Ash Meadows aquatic endemic organisms for the most part rely on climax communities, while slow stream velocities and silt-laden substrates favor non-native species such as mollies and crayfish. Such conditions provide unsuitable habitat for endemic invertebrates, and alter community dynamics and nutrient transport to the pupfish. They also physically place pupfish, especially their eggs and larvae, in close proximity to the non-native predators. Other emergent vegetation, such as bulrush (*Scirpus* sp.), also creates these undesirable habitat conditions, albeit to a lesser extent. Service staff have been involved with several efforts to remove cattail from pupfish habitat but even these effort only offers a temporary respite as cattail reinvade areas from which they were removed.

Terrestrial Weeds

Terrestrial habitats provide a source of allochthonous material critical for aquatic production. In many streams, a majority of the energy comes from invertebrates and vegetation that fall in the stream. For example, at Devils Hole, terrestrial vegetation provided 1.3 times more energy than internal aquatic algae, and terrestrial insects 2.7 times more energy than aquatic invertebrates. In addition, allochthonous carbon was over six times greater than autochthonous carbon (Blinn and Wilson 2004). In general, a variety of allochthonous material available to streams provides a wide range of nutrients, supporting a greater diversity of organisms requiring specific food sources that are used as prey by the pupfish.

Over 63 non-native plant species have been identified on the Refuge (Service 2006). Many of these species are noxious agricultural weeds such as Russian knapweed, five hook bassia (*Bassia hyssopifolia*), Maltese star-thistle (*Centaurea melitensis*), yellow star thistle (*C. solstitialis*), and hoary cress (*Cardaria draba*) (Service 2006). Non-native plant species directly compete with native species for water, nutrients and sunlight. Many invasive species have allelopathic adaptations that allow them to exclude and out-compete native vegetation and colonize previously undisturbed habitat. Non-native species can also indirectly affect native species by changing ecosystem processes such as nutrient cycling and fire regimes, which have altered the input of allochthonous materials into pupfish habitat. Alteration of these processes has been shown to change chemical composition of the water, such as pH in the Jackrabbit Spring outflow following the 2005 Meadows Fire (Shawn Goodchild, Service, Fish and Wildlife Biologist, personal observation, 2005), as well as available diversity of food resources and nutrients (Kennedy et al. 2004).

The fire history of Ash Meadows is not well known. Although fire has always been naturally occurring, and it is probable that Native Americans used fire to burn riparian areas during the human history of Ash Meadows (Kathleen Sprowl, Archaeologist, Bureau of Land Management – Las Vegas Field Station, personal communication, 2007), fire facilitated and intensified by non-native species is a new threat to pupfish not identified in the original listing. Fire has been shown to be a threat to endemic fishes at the Refuge. For example a fire in the Jackrabbit Spring system eliminated fish from inside and downstream of the footprint of the fire. Fires in riparian areas affect pupfish in three major ways. Fire increases temperature in the stream, and in thermal springs it often increases temperatures beyond the organism's thermal limits. This occurs from heat generated by the fire, as well as subsequent lack of shading. Inputs of ash and other debris rapidly changes water chemistry such as nutrients and pH, often beyond tolerance of the aquatic organisms (Earl and Blinn 2003). Fire also alters allochthonous nutrients, which disrupts the aquatic food web until native vegetation regenerates. A sudden influx of nutrients and eroded materials from burned sites exponentially increases autochthonous production, causing oxygen depletion and physical smothering of substrate. Non-native plant species are known to change fire regimes and are a threat to biodiversity (Brooks et al. 2004). In some areas of the Refuge, non-native salt cedar (*Tamarix* sp.) and annual weeds appear to be spreading fire through riparian corridors, as well as along the spring channels and in other pupfish habitat. Three major fires in 2004 and 2005 (the Meadows Fire, Longstreet Fire and Ash Fire) appeared to be spread through stands of salt cedar, however, the relationship between weeds and fire at the Refuge needs to be studied in more detail. In other ecosystems where non-native weeds have increased fire frequencies, this has led to reductions in native plant cover and diversity (Brooks et al. 2004). Where there are weeds, anecdotal observations suggest fire provides an opportunity for non-native plants to expand on the Refuge. Following the Meadows Fire, Russian knapweed populations were competitively released and rapidly expanded to create monocultures that now likely prevent regrowth and colonization of native vegetation (Baldino 2006a). However, on sites where weeds are not present, more frequent fire may be beneficial to pupfish habitat in maintaining low fuel densities, and thereby preventing infrequent catastrophic fires.

The Refuge is addressing the terrestrial weed problem, and recently completed an Integrated Pest Management Plan (IPM). This IPM outlines control strategies and methods for managing invasive species on the Refuge and is the Refuges' long-term approach for eradicating and managing invasive species on the Refuge. The IPM includes mapping, monitoring, and incorporates restoration planning and best management practices, and approaches weed management in the context of habitat restoration. There is no long-term funding to implement this IPM; however, in 2006, the Refuge received five years of funding through the Southern Nevada Public Land Management Act for weed mapping and eradication. While significant, this is only a fraction of the funding required to implement the IPM and will not address the threat posed by weeds present in the thousands of acres of abandoned agricultural fields adjacent to pupfish habitat.

Invasive plant species will continue to be a threat for the foreseeable future and will require regular management and monitoring. Pupfish habitat is extremely vulnerable to non-native species. Left untreated, weed infestations will continue to result in increased fire intensities and/or frequencies, which will inevitably lead to decreases in the population of Ash Meadows Amargosa pupfish. While the IPM now provides a framework to manage invasive species on the Refuge, these efforts are just beginning to be implemented and in many areas weed cover continues to expand. Based on this information, we conclude the magnitude of the threat from weeds is high and the immediacy to be imminent.

Other Anthropogenic Factors

The listing rule identifies vandalism as a natural or manmade factor threatening the pupfish and states that local populations may be extirpated as a result. Given the establishment of the Refuge and law enforcement presence, the risk of vandalism is much less than when the pupfish was listed. This may not be true for populations that occur on private property, such as the 'Spring at Clay Pit' population; however, it is likely that this population has been extirpated and it consisted of a very small proportion of the entire species numbers. Based on this information, we conclude the magnitude of the threat is low and the immediacy to be non-imminent.

Stochastic Events

Small populations have an inherent risk of extirpation due to stochastic and catastrophic events. Fire and flooding are natural catastrophic events that occur within the pupfish's range, that are exacerbated by manmade factors such as weeds, unnatural ignition of fires, and altered hydrology. The geographic isolation of small populations also increases the chance of extirpation of metapopulational segments due to stochastic events. For example, a fire that affects a small isolated spring outflow has a greater chance of extirpating the pupfish in that system than would one that occurs in a more complex, larger stream that has a greater number of individuals in the population. Due to the widespread nature of pupfish, a catastrophic event in one location would not impact the entire population. Based on this information, we conclude the magnitude of the threat from stochastic events is moderate and the immediacy to be non-imminent.

Limited Habitat Size

The pupfish has survived since the Pleistocene within a small home range. The listing rule identifies the small range of the pupfish as a threat, and currently, this is the circumstance considering habitat alterations have isolated subpopulations of the pupfish and/or made specific habitats unsuitable. Habitat disturbance has also prevented genetic flow between subpopulations, and it is unclear as to how this affects the overall genetic health of the species. In some cases, habitat characteristics alter phenotypical characteristics of the species, which could be reflected in loss of specific alleles. Prevention of gene migration between subpopulations may cause alleles to be lost permanently. As previously discussed, studies with mitochondrial DNA suggest that there have been bottlenecks in some populations; however not as much as what has occurred with other subspecies. These bottlenecks are a consequence of small habitats, and not necessarily dependant on anthropogenic disturbance. Another danger of small home ranges is the threat of destruction from a catastrophic event, such as a natural event, such as flooding or fire; or a human-caused event, such as groundwater withdrawal or a chemical spill. Based on this information, we conclude the magnitude of the threat from limited habitat size is low and the immediacy to be non-imminent.

III. RECOVERY CRITERIA

Recovery plans provide guidance to the Service, States, and other partners and interested parties on ways to minimize threats to listed species, and on criteria that may be used to determine when recovery goals are achieved. There are many paths to accomplishing the recovery of a species and recovery may be achieved without fully meeting all recovery plan criteria. For example, one or more criteria may have been exceeded while other criteria may not have been accomplished.

In that instance, we may determine that, over all, the threats have been minimized sufficiently, and the species is robust enough, to downlist or delist the species. In other cases, new recovery approaches and/or opportunities unknown at the time the recovery plan was finalized may be more appropriate ways to achieve recovery. Likewise, new information may change the extent that criteria need to be met for recognizing recovery of the species. Overall, recovery is a dynamic process requiring adaptive management, and assessing a species' degree of recovery is likewise an adaptive process that may, or may not, fully follow the guidance provided in a recovery plan. We focus our evaluation of species status in this 5-year review on progress that has been made toward recovery since the species was listed (or since the most recent 5-year review) by eliminating or reducing the threats discussed in the five-factor analysis. In that context, progress towards fulfilling recovery criteria serves to indicate the extent to which threat factors have been reduced or eliminated.

The approved Ash Meadows Recovery Plan is ecosystem based and is not specific to the five listing factors. Included in Table 2 are the downlisting criteria, and in Table 3 the delisting criteria, which are relevant for the Ash Meadows Amargosa pupfish described in the Ash Meadows Recovery Plan.

Table 2: Ash Meadows Recovery Plan Downlisting Criteria Relevant for Ash Meadows Amargosa Pupfish.

1. All non-native animals and plant species must be eradicated from essential habitat. These non-native species currently include sailfin mollies, mosquitofish, largemouth bass, black bullheads, bullfrogs, crayfish, turban snails, wild horses, salt cedar, and Russian olive.

2. Secure and protect the Ash Meadows aquifer so that all spring flows return to historic discharge rates, and the water level in Devils Hole is maintained at a minimum level of 1.4 feet below the copper washer. Spring discharge rates will be determined by Task number 211.

3. Reestablish water to historic springbrook channels that are free of barriers that eliminate genetic exchange between populations by preventing movement of native fishes throughout their historic range.

4. The essential habitat must be secure from detrimental human disturbances including mining, off-road vehicles, and introduction of non-native species.

5. All listed fish species are present in all the springs that they have occupied historically as identified in Appendix A, Table XIII.

Table 3: Ash Meadows Recovery Plan Delisting Criteria Relevant for the Ash Meadows Amargosa Pupfish.

1. Criteria shown above (Table 2) for downlisting from endangered to threatened.

3. Native plant communities and aquatic communities have been reestablished to historic structure and composition within all essential habitat.

4. Each individual spring or stream population of Warm Springs pupfish, Ash Meadows Amargosa pupfish, and Ash Meadows speckled dace have sex ratios and juvenile-to-adult ratios that support self-sustaining populations as determined by Task 626.

Ash Meadows Recovery Plan Downlisting Criteria Narrative for Ash Meadows Amargosa Pupfish.

1. All non-native animals and plant species must be eradicated from essential habitat. These non-native species currently include sailfin mollies, mosquitofish, largemouth bass, black bullheads, bullfrogs, crayfish, turban snails, wild horses, salt cedar, and Russian olive.

This criterion is influenced by three of the five listing factors: 1) Present or threatened destruction, modification, or curtailment of a species habitat or range, 2) disease or predation, and 3) other natural or man-made factors affecting continued existence. This criterion has not been met, and is currently infeasible given the extent of non-native species and the difficulties inherent with environmental conditions. Some species identified by the plan have been removed. In 2007, black bullhead (*Ameiurus melas*) was eradicated from Ash Meadows using the fish toxicant rotenone. Wild horses and burros were removed and excluded from the Refuge in 1995. All other non-native species referenced in the recovery plan are unlikely to be eradicated due to a wide distribution, large numbers, habitat preferences, cohabitation with listed species, or resistance to removal methods. Substrate-dwelling species such as turban snails, crayfish, and bullfrogs are likely impossible to eradicate other than with drying, persistent chemicals, or other drastic measures that would be detrimental to pupfish. Sailfin mollies and mosquitofish are both interwoven with populations of pupfish, occur in dense vegetation, and are resistant to standard removal methods such as rotenone.

Several non-native plant species have been introduced or have moved on to the Refuge that were not identified in the recovery plan, including a multitude of terrestrial weeds, primarily Russian knapweed, Maltese star-thistle, and five-hook bassia. Eradication of non-native terrestrial species is important as the weeds lower biodiversity of native plants, which negatively influences the quality of allochthonous inputs into the aquatic system. In addition, weeds such as *Bassia* overgrow channels creating physical barriers, preventing energy inputs from a diversity of allochthonous materials, as well as solar energy. These physical barriers also capture debris, causing water to overflow banks and allow pupfish to enter unnatural, unstable habitats.

Additional non-native aquatic species that have been identified in pupfish habitat since the development of the recovery plan include green sunfish and convict cichlid (*Archocentrus nigrofasciatum*), the latter of which were eradicated in 2008. Both of these species have similar effects as the younger life stages of largemouth bass, but impact native species in a wider variety of habitats, since they are adapted to a wider range of flow characteristics.

Earlier modifications of habitat for agriculture throughout Ash Meadows continue to curtail pupfish habitat (i.e. from weeds) and range (i.e. from predatory fish) by the continued existence of channelized springs, abandoned fields, and roads that are in early successional stages. These conditions also enhance the survival and competitive advantage of non-native organisms. These modifications also create a mosaic of habitat patches where listed species occur in low numbers, making activities, such as spraying herbicide under guidance of an approved Pesticide Use Plan, difficult under existing regulations. In addition, the increasing occurrence of non-native species may elevate parasitism rates in pupfish. Predation, especially of larval pupfish, may also increase. Finally, other natural or manmade factors, as identified in the Federal Register (48 FR 610) as being the introduction of exotic fish and snails, influences this criterion. Continued introductions have occurred, such as the convict cichlid. Other non-native species are being found, such as the ramshorn snail (*Planorbella* sp.). This criterion is being addressed at several levels as described in the Recovery Plan. Tasks 222 and 232 regarding weed and non-native aquatic organisms are ongoing. These involve developing and implementing acceptable control options. Task 223, removal of wild horses, has been accomplished.

2. Secure and protect the Ash Meadows aquifer so that all spring flows return to historic discharge rates, and the water level in Devils Hole is maintained at a minimum level of 1.4 feet below the copper washer. Spring discharge rates will be determined by Task number 211.

One listing factor is relevant to this criterion: the present or threatened destruction, modification, or curtailment of its habitat or range. Loss of groundwater resources may limit aboveground flow, ultimately reducing volume of water occupied by pupfish. The amount, distribution, and suitability of pupfish habitat are dependent on both surface and subsurface hydrologic features. Surface water elevation in Devils Hole indicates the elevation of the groundwater table and is an indicator of potential changes in spring flows that may affect Ash Meadows Amargosa pupfish. Water levels in Devils Hole, as monitored by the National Park Service, are experiencing a gradual, long-term decline in water level at approximately 6-9 millimeters per year (Harrill and Bedenger 2005). In addition, several monitoring stations have been implemented by both the Service and USGS (i.e. USGS 2006).

Task 1 of the Recovery Plan identifies actions to secure and protect the aquifer. Recovery Task 111 requires determination of historic spring flow channels and discharge rates. Several efforts have been completed to accomplish this task, including hydrological surveys to map present and historic water channels; however, adaptive monitoring needs to be continued to maintain and refine information. Tasks 114 and 122 to secure and protect groundwater rights within and outside of the Ash Meadows area are ongoing and opportunistic.

3. Reestablish water to historic springbrook channels that are free of barriers that eliminate genetic exchange between populations by preventing movement of native fishes throughout their historic range.

One listing factor is relevant to this criterion: the present or threatened destruction, modification, or curtailment of its habitat or range. Nearly all habitat for the pupfish at Ash Meadows has been modified, thereby influencing their range. In most cases, the historic springbrook channel no longer exists, and in some cases cannot be re-created because the slope has been altered or, as with the Carson Slough, the route the water is supposed to take would put it through private property. It is difficult to determine if range has physically expanded, since pupfish occur in shallow marshes created by degraded agricultural infrastructure. In addition, concrete ditches extend suitable habitat beyond what soil-lined streams would naturally provide. These unnatural water courses provide a poor quality habitat, and support less pupfish than would a natural, native system. They may be more expansive in area, but contain a lower density of pupfish than a smaller area of quality habitat. A qualifier should be developed to define high- and low-quality areas.

Several restoration projects have been completed and/or are ongoing. These include Crystal Spring, Kings Pool, Point-of-Rocks streams, and Jackrabbit outflow. Several more are planned, including lower Jackrabbit outflow, Big Springs and outflow, Crystal Reservoir, and Carson Slough with step down restorations such as Fairbanks Spring. Given the prevalence of non-native species, intra- and/or inter-stream barriers are necessary to limit range and movement of predaceous species, which have not been addressed in the Recovery Plan. As habitat is modified to limit non-native species, and other non-native species removals are successful, assisted migration may be implemented in the short term and in the long term seasonal or permanent connections between restored channels would be restored.

Task 21 of the Recovery Plan identifies actions to identify historic systems, and develop and implement restoration plans. Task 22 identifies actions to enhance and restore terrestrial systems, and Task 23 identifies actions to enhance and restore aquatic systems. Currently, Task 21 has been completed; however, the products (plans) need ongoing adaptation to enhance precision. Tasks 22 and 23 are ongoing.

4. The essential habitat must be secure from detrimental human disturbances including mining, off-road vehicles, and introduction of non-native species.

The two listing factors relevant to this criterion are: 1) The present or threatened destruction, modification, or curtailment of its habitat or range, and, 2) other natural or manmade factors affecting its continued existence. These specifically include habitat disturbance, potential for mineral extraction, and introduction of non-native species. Since the listing of the species, most of the habitat occupied by the pupfish has been acquired by the Refuge, or is BLM land. This provides some level of control to unregulated detrimental human disturbances. The anticipated transfer of land from BLM to the Service, as well as the minerals withdrawal, has not been completed, therefore regulation does not yet preclude future mining. Off-road vehicles have been limited by regulation, barriers, and law enforcement, but still occur at low frequencies. Some habitat, such as at the springs at Clay Pits and Five Springs, occur on private property, and are not subject to the same Federal regulations that protect habitat on the Refuge and BLM properties. Non-native species introductions still occur (i.e. convict cichlid, goldfish/koi); however, they are likely minimized due to these same factors. Several parcels of private property lie within the Refuge, and contain noxious weeds that spread onto Refuge property. In addition, landowners can potentially introduce non-native aquatic organisms on their property, such as game fish or crayfish. Non-intentional introductions from stream connections to private property are still possible, such as largemouth bass in Big Springs, but these situations are currently being addressed. In addition, several landowner cooperation projects are ongoing, with the Service's Partners Program, which will enhance pupfish habitat on or adjacent to private property.

Task 24 of the Recovery Plan requires minimization of human disturbance. This is an ongoing Task. Other Recovery Tasks facilitate this criterion, specifically Task 25, monitoring, which allows for early detection of non-native species.

5. All listed fish species are present in all the springs that they have occupied historically as identified in Appendix A, Table XIII.

The listing factor relevant to this criterion is the present or threatened destruction, modification, or curtailment of its habitat or range. Loss of fish in these habitats represents a range reduction; however, it is unclear as to whether or not some of these habitats were historically ideal for the species.

Pupfish are present in all twenty-one historic habitats described in Appendix A, Table XIII of the Recovery Plan, except for Crystal Reservoir, Bradford #1, Tubbs, and Forest Spring. Bradford #1 and Forest Spring are too cool to be ideal pupfish habitat; however, they are connected to occupied habitat and pupfish could move into them if conditions allowed. Currently capturing or observing a pupfish in these two springs is extremely rare. Tubbs Spring is isolated due to a pipe; however, speckled dace have navigated the pipe from occupied habitat and it is possible pupfish could do the same. Surveys in Tubbs Springs on October 2, 2006, indicated Tubbs Spring was infested with crayfish and no pupfish were captured. The site described as Spring at

Clay Pits is on private property and the status of this population is unknown. In recent conversations with Refuge staff, the landowner stated he recently filled in the springs and built build a racetrack. Crystal Reservoir contains non-native predators (green sunfish and largemouth bass), that extirpated pupfish from the reservoir. The reservoir is connected to occupied habitat, and would be re-inoculated with pupfish from the Crystal Spring ditch if predatory fish were removed; however it currently functions as a population sink for pupfish emigrating to the reservoir. The Point-of-Rocks pools have been removed and pupfish are now abundant in the several springs in this area. Soda Springs has diminished in flow, and is now overgrown with emergent vegetation. Pupfish had been considered extirpated; however, two individual pupfish have been observed within the spring during February 2008.

Task 234 is to reestablish the four listed fish, including the Ash Meadows Amargosa pupfish, throughout their historic range. This task is ongoing, however, pupfish have been extirpated from only a few sites and restoration has allowed for emigration of pupfish into previously unoccupied habitat. Other sites, such as Soda Springs, have not been restored to allow for reestablishment.

Ash Meadows Recovery Plan Delisting Criteria for the Ash Meadows Amargosa Pupfish

1. Criteria shown above (Table 2) for downlisting from endangered to threatened.

Factors for Criterion 1 are discussed above.

3. Native plant communities and aquatic communities have been reestablished to historic structure and composition within all essential habitats.

Native plant communities and aquatic communities have been reestablished to historic structure and composition at very few sites. The upper Kings Pool area has been restored to historic structure, and the Point of Rocks and Jackrabbit Stream are ongoing restorations. The upper portion of Crystal Stream has been restored, but needs additional work to narrow the channel. Invasive weeds, including cattails, and non-native aquatic species have compounded problems with community reestablishment, and represent a major threat to pupfish communities.

Task 21 of the Recovery Plan identifies actions to identify historic systems, and develop and implement restoration plans. Task 22 identifies actions to enhance and restore terrestrial systems, and Task 23 identifies actions to enhance and restore aquatic systems. Currently, Task 21 has been completed; however, the restorations require periodic manipulations to maximize their suitability as pupfish habitat. Tasks 22 and 23 are ongoing.

The two relevant listing factors are: 1) present or threatened destruction, modification, or curtailment of its habitat or range, and 2) other natural or manmade factors affecting its continued existence. Existing modified habitats support disrupted ecologic communities and ecological succession of these habitats typically further damage these communities, such as by choking a thermal stream with cattails. Introductions of non-native species and off-Refuge activities (such as development of inholdings) in historic habitat alter community profiles of species, and vandalism, typically from illegal swimming, is an ongoing impact. Effects of activities on habitats outside of Refuge boundaries are unknown, but likely continue to influence the pupfish if they are still present.

4. Each individual spring or stream population of Warm Springs pupfish, Ash Meadows Amargosa pupfish, and Ash Meadows speckled dace have sex ratios and juvenile-to-adult ratios that support self-sustaining populations as determined by Task 626.

The major springs in Ash Meadows have been monitored since 1989, and have been considered to have self-sustaining populations of pupfish. Small mesh traps are used to capture all size classes to determine size ratios of fish using Length-Frequency models. Sex ratios are not determined, as it is not possible to determine sustainability of populations without knowledge of effective population size and genetics. It is also difficult to determine gender in smaller fish, and attempts to do so may cause undue stress and injury to the fish. Some habitat parameters have been developed for the pupfish, as discussed later.

Task 23 identifies actions to enhance and restore aquatic systems, including reestablishing populations. Task 25 identifies monitoring reestablished/enhanced populations. Both of these Recovery Tasks are ongoing, biennially as identified in the Recovery Plan as well as opportunistically during non-native species removal efforts and in support of other projects.

Information derived from these tasks informs management actions to restore pupfish habitat; however, a natural or manmade factor affecting the species' continued existence, i.e., low population numbers, could affect parameters that influence the population. For example, low populations may have a skewed effective population size that would have additional negative impacts on the overall genetic variability of the population. Low genetic variability could negatively influence sustainability of the population, especially during catastrophic events.

IV. SYNTHESIS

Section 3 of the Act defines an "endangered species" as "an animal or plant species in danger of extinction throughout all or a significant portion of its range." Threats to the species occur in high, moderate, or low magnitudes as defined in 48 FR 43098-43105. Many threats to the Ash Meadows Amargosa pupfish as discussed in the original listing, including local groundwater extraction on the Refuge, habitat destruction by cattle and wild horses, and OHV activity, have largely been addressed by the designation of Ash Meadows as a National Wildlife Refuge, purchase of land and water rights, and through BLM activities such as wild horse removal.

Two threats under listing Factor A, habitat degradation and invasive non-native species, continue to be the most important obstacles to long-term protection and delisting of the pupfish. One threat, the off-Refuge development of groundwater from the carbonate and basin-fill aquifers that supply Ash Meadows, has the potential of seriously limiting available habitat. Prior to a change in status these threats to the pupfish must be managed. In this evaluation, we determined the threat posed by groundwater withdrawal is high and non-imminent, the threat posed by aquatic invasive non-native species is high and the immediacy is imminent, and the threat posed by surface mining is of low magnitude and non-imminent immediacy. The decline in groundwater levels due to groundwater pumping has not been abated but is currently being monitored and legal protection for the water that supports the Devils Hole pupfish appears sufficient to protect the Ash Meadows Amargosa pupfish; however, additional monitoring of springflow and groundwater needs to be implemented and contingency plans should be developed.

The threat posed by invasive species presents a major challenge. Non-native plant and animal species pose a threat to the pupfish through direct predation, competition, exclusion, by changing ecosystem processes such as fire, and simplifying allochthonous inputs. Fire fueled by non-native plant species is a new threat to the pupfish not described in the 1985 listing rule. Non-native species previously confined to abandoned agricultural fields now appear to be moving out into riparian habitat. The Refuge is making significant strides in addressing the threat posed by non-native species, including the recent completion of the IPM Plan and securing short-term funding for salt cedar and other non-native species removal. However, these activities have only recently been initiated, there are no tangible on-the-ground results that demonstrate the threat to the pupfish has been reduced, and there is no long-term funding in place to ensure progress can continue after initial efforts.

Anecdotal observations suggest the pupfish is a pioneer species, and populations increased largely due to natural recovery after groundwater pumping and disturbances (agriculture, wild horse grazing, and OHV activity) were discontinued on the Refuge. However, there are no data or information to quantify population increases. The Recovery Plan describes five downlisting criteria for the pupfish. Since approval of the Recovery Plan in 1990, only two (#2 and #3) of these criteria have been partially completed. As described in the downlisting criteria, populations should be mapped and demographic population data collected. A firm understanding of population demographics and population trends for the pupfish is necessary before conclusions regarding recovery can be made.

Given the seriousness of the threat posed by invasive species, the high threat from groundwater development, lack of quantitative information to determine recovery, and the downlisting criteria that remain incomplete, it is our conclusion that a change in status for the Ash Meadows Amargosa pupfish from endangered is not warranted at this time and the pupfish continues to meet the definition of an endangered species. The Ash Meadows Amargosa pupfish is currently assigned a listing priority number of 15. A number 15 reflects a low degree of threat with a high potential for recovery for a subspecies. Under Service guidance "a species with a low degree of threat is rare or is facing a population decline which may be a short-term, self correcting fluctuation, or the impacts of threats of the species habitat are not fully known"(48 FR 43098-43105). A moderate degree of threat indicates "the species will not face extinction if recovery is temporarily held off, although there is continual population decline or threat to its habitat" (48 FR 43098-43105). Based on this guidance, we conclude that the pupfish is subject to a moderate degree of threat, instead of the prior low degree of threat.

Under Service guidelines, recovery potential is classified as either high or low. A high potential for recovery means the biological and ecological limiting factors are well understood, threats to the species' existence are well understood and easily alleviated, and intensive management is not needed. Based on the level of natural recovery observed on the Refuge to date and the life history of the pupfish, we believe the species will require ongoing management to restore habitat and manage for non-native species on the Refuge. Some issues affecting the pupfish, such as non-native species removal and groundwater reduction are not easily alleviated; however, it is unlikely that the springs will significantly diminish in flow within the next five years. Removal plans target the aquatic invasive species that have the most impact. Recovery of the species will be facilitated by the IPM Plan that provides a management framework and process for managing non-native species on the Refuge, and the Ash Meadows Geomorphic and Biological Assessment that provides a framework for future restoration activities. For these reasons, we conclude the Ash Meadows Amargosa pupfish is best described as having a high recovery potential.

V. RESULTS

Recommended Listing Action:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist (indicate reason for delisting according to 50 CFR 424.11):
  ____ *Extinction*
  ____ *Recovery*
  ____ *Original data for classification in error*
__X__ No Change

New Recovery Priority Number and Brief Rationale:

Listing and Reclassification Priority Number and Brief Rationale: The Ash Meadows Amargosa pupfish is currently assigned a listing priority number of 15. In determining the listing priority number, both the degree of threats and potential for recovery are considered. A number 15 reflects a low degree of threat with a high potential for recovery as applied to a subspecies. As described above, the pupfish is subject to a moderate level of threats but has a high potential for recovery as a subspecies. ***Therefore, we recommend applying the Recovery Priority Number 9.***

VI. RECOMMENDATIONS FOR ACTIONS OVER THE NEXT 5 YEARS

The Recovery Plan is ecosystem based and describes recovery actions that benefit all Refuge species. The Ash Meadows Amargosa pupfish could be considered for a change of status in the near future; however, several steps or actions are still needed before downlisting or delisting can take place.

Recommendations include:

- The Recovery Plan should be updated using the most recent and best scientific and management information available.

- Future genetics work needs to be completed in more detail for the species and included in an updated Recovery Plan, including a genetics management plan if deficiencies in alleles from anthropogenic impacts are detected.

- Predatory, non-native fish, which prey on adult and juvenile pupfish, should be prioritized for removal. Restorations should be based on limiting habitat for non-native fish and invertebrates. Removal of reservoirs to eradicate largemouth bass and green sunfish is essential to the downlisting of pupfish, as is reduction of populations of sailfin mollies, crayfish, and Mosquitofish.

- The Refuge is implementing many restoration projects that could benefit the pupfish; however, minimal effectiveness monitoring specific to the pupfish and other endemic species is conducted. To document recovery of the pupfish, these projects should include pre- and post-site sampling to verify and quantify that restoration actions are benefiting the species.

- Non-native weeds are a major threat to the pupfish, and the IPM Plan is an important step towards addressing this problem. Refuge staff have been very successful in securing short-term funds to begin implementing the IPM Plan. However, the non-native weed problem is a continuing threat that will require long-term funding. Prior to delisting, long-term funding should be secured for non-native species control on the Refuge.

- Surface mining remains a threat to the pupfish. Prior to downlisting, Service and BLM lands with public minerals must be withdrawn from mining claims. Unless these mineral rights are purchased or transferred to the Service, a program needs to be established to renew the mineral withdrawal every 20 years before downlisting can occur.

- Crystal Reservoir, Bradford Spring, and Forrest Spring should be removed from the list of areas to be protected prior to downlisting of the pupfish. Crystal Reservoir is an artificial impoundment, and Bradford and Forrest Springs are too cool to be ideal pupfish habitat. Management of Bradford and Forrest Springs would remain as downlisting criteria for the Ash Meadows speckled dace (*Rhinichthys osculus nevadensis*).

- The downlisting requirement that all non-native species be removed should be updated, and limited to complete removal of centrarchids, cichlids, and other similar predatory fish. It is infeasible to remove 100 percent of all non-native fish, invertebrates, or plants, and efforts should be directed both towards habitat restoration poorly suited to non-native species, as well as targeted removals to fulfill specific management purposes.

- As more information is gained regarding habitat preference of pupfish and non-native species, qualifiers should be developed to construct more specific recovery guidelines based on habitat restoration.

- Additional life history work should be funded and implemented for the endemic aquatic species of Ash Meadows. Much of the life history work for Ash Meadows Amargosa pupfish has been developed from populations in the spring pools, and species recovery would benefit from knowledge derived from their other communities. This could also involve academia, zoos, and aquarists in determining propagation guidelines, need for refugia, and other information pertinent to the pupfish.

- Information and educational activities should be a focus in recovery efforts. An informed public would take additional ownership regarding the pupfish and associated issues with the pupfish. This would help create a clear link between resource-use, such as groundwater, and the environment, which may help foster environmental stewardship. This could also involve work with zoos, aquarists, and academia.

- Fuel breaks should be identified, incorporated into the Refuge Fire Management Plan, IPM and restoration plans, and implemented to help prevent catastrophic fire events that could significantly affect pupfish populations.

- Studies should be implemented to identify relationships between fire and weeds.

- Upland and riparian restoration should be designed to include the greatest variety of native plant species as possible, both to increase diversity of allochthonous inputs as well as to minimize erosion.

- Dams and dikes should be removed, including reservoirs and upland water diversion structures, in order to facilitate substrate flow.

- Property and/or rights, such as conservation easements, should be acquired on private property that occur within pupfish habitat, or could affect restoration of pupfish habitat.

- Establish regular coordination with owners of property adjacent or within pupfish habitat to prevent and/or eliminate non-native species introductions, primarily aquatic species and noxious weeds, should occur.

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U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW

Ash Meadows Amargosa Pupfish (*Cyprinodon nevadensis mionectes*)

Current Classification: Endangered

Recommendation Resulting from the 5-Year Review:

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

Appropriate Listing/Reclassification Priority Number: 9

Review Conducted By: ___Paul Barrett, Fish and Wildlife Biologist___________

FIELD OFFICE APPROVAL:

Lead Field Supervisor, U.S. Fish and Wildlife Service

Approve ______________________________ Date 3/17/10