CHIRICAHUA LEOPARD FROG
(Lithobates [=*Rana*] *chiricahuensis)*

5-Year Review:
Summary and Evaluation

Photo by Jim Rorabaugh

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

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5-YEAR REVIEW

Chiricahua Leopard Frog (*Lithobates* [=*Rana*] *chiricahuensis*)

1.0 GENERAL INFORMATION

1.1 Reviewers:

Lead Regional Office: Southwest Regional Office, Region 2
Susan Jacobsen, Chief, Threatened and Endangered Species 505-248-6641
Wendy Brown, Recovery Coordinator, 505-248-6664
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Lead Field Office: Arizona Ecological Service's Tucson Suboffice
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Cooperating Field Office: New Mexico Ecological Services Office
Michelle Christman, Fish and Wildlife Biologist 505-346-2525 x4728

1.2 Methodology used to complete the review:

The U.S. Fish and Wildlife Service (USFWS) conducts status reviews of species on the List of Endangered and Threatened Wildlife and Plants (50 CFR 17.12) as required by section 4(c)(2)(A) of the Endangered Species Act (ESA) (16 U.S.C. 1531 *et seq.*). The public notice for this review was published in the *Federal Register* on April 23, 2007 (72 FR 20134).

This review considers both new and previously existing information from the recent Chiricahua Leopard Frog Recovery Plan (USFWS 2007), publications and reports produced since the recovery plan was prepared, and the latest monitoring data. The recovery plan was developed by a recovery team that consisted of both a technical team of experts on the frog, its habitats, and conservation biology, and also of three stakeholder groups, made up of landowners and managers, ranchers, and representatives of environmental groups, mining companies, Tribes, wildlife agencies, and other interested or affected parties. The planning process included a Nature Conservancy-style threats assessment by recovery unit and a Population and Habitat Viability Analysis facilitated by the IUCN's Conservation Breeding Specialist Group.

Note that we use the scientific name *Lithobates chiricahuensis* for this taxon, which is consistent with the latest Society for the Study of Amphibians and Reptiles list of scientific and common names (Crother 2008). However, the species was originally listed as *Rana chiricahuensis*. The arrangement put forth by Crother has been challenged (Pauly *et al.* 2009) and may not stand. Nevertheless, we recommend the new scientific name of *Lithobates chiricahuensis* be adopted for this taxon.

In addition, we recommend that the Ramsey Canyon leopard frog (*Lithobates subaquavocalis*), be subsumed into *L. chiricahuensis*, as supported by Crother (2008) and noted by the USFWS as part of the listed entity in a 90-day finding on 192 species from a petition to list 475 species (74

FR 66867; December 16, 2009) (see *Genetics, phylogeny, and taxonomy* below). The recovery plan treated the Ramsey Canyon leopard frog as the Chiricahua leopard frog under the assumption that it would soon be recognized as the same species.

This 5-year review was sent to the Nongame Herpetological Program at the Arizona Game and Fish Department, Phoenix, Arizona for review; however, no comments were received.

1.3 Background

1.3.1. FR Notice citation announcing initiation of this review: 72 FR 20134

1.3.2 Listing history

<u>Original Listing</u>
FR notice: Federal Register 67(114):40790-40811
Date listed: June 13, 2002
Entity listed: *Lithobates* (*Rana*) *chiricahuensis*
Classification: Threatened without critical habitat

1.3.3 Associated rulemakings: None. However, a proposed critical habitat designation is in preparation and is expected to publish in the Federal Register in March 2011, with a final rule expected by 2012.

1.3.4 Review History: No other 5-year reviews have been prepared for this species. The recovery plan was finalized in April 2007.

1.3.5 Species' Recovery Priority Number at start of 5-year review: 2C.
A Recovery Priority Number of 2C is indicative of a taxon with a high degree of threat, a high recovery potential, and the taxonomic standing of a species. The C indicates that the species' recovery conflicts with water demands, development projects, or other forms of economic activity.

1.3.6 Recovery Plan

Name of plan: Chiricahua Leopard Frog (*Rana chiricahuensis*) Recovery Plan
Date issued: April 2007
Dates of previous revisions, if applicable: Not applicable

2.0 REVIEW ANALYSIS

2.1 Application of the 1996 Distinct Population Segment (DPS) Policy:

2.1.1 Is the species under review a vertebrate? Yes.

2.1.2 Is the species under review listed as a DPS? No.

2.1.3 Is there relevant new information for this species regarding the application of the DPS policy? No.

2.2 Recovery Criteria

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

2.2.2 Adequacy of recovery criteria

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

2.2.2.2 Are all of the 5 listing factors that are relevant to the species addressed in the recovery criteria? Yes.

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

Recovery Criteria: To delist the Chiricahua leopard frog, the following recovery criteria must be achieved:

Criterion 1. At least two metapopulations located in different drainages (defined here as USGS 10-digit Hydrologic Units) plus at least one isolated and robust population occur in each recovery unit (RU) that exhibit long-term persistence and stability (even though local populations may go extinct in metapopulations) as demonstrated by a scientifically acceptable population monitoring program. Interpreting the results of the monitoring program will take into account precipitation cycles of drought or wet periods and the effects of such cycles on population persistence.

Criterion 2. Aquatic breeding habitats, including suitable, restored, and created habitats necessary for persistence of metapopulations and robust isolated populations identified in criterion 1, are protected and managed in accordance with the recommendations in this plan.

Criterion 3. The additional habitat needed for population connectivity, recolonization, and dispersal is protected and managed for Chiricahua leopard frogs, in accordance with the recommendations in this plan.

Criterion 4. Threats and causes of decline have been reduced or eliminated, and commitments for long-term management are in place in each RU such that the Chiricahua leopard frog is unlikely to need protection under the ESA in the foreseeable future.

For the purposes of delineating habitat and clarifying recovery, the eight Recovery Units (RUs) are defined from the USFWS 2007 Recovery Plan as: “natural units in which frog metapopulation dynamics function or could function as the species recovers.” The RUs cover the entire known range of the species in Arizona, New Mexico, and adjoining portions of Mexico, which ensures that when recovered, the frog will be well-represented throughout its present and historical range (See Figure 1). Recovery Units 1-4 cover the southern populations, while RUs 5-8 contain the Mogollon Rim populations. Each unit is large enough to ensure that frog carrying capacity is buffered against changes due to potential successional processes or environmental disasters (e.g. floods, fire, drought, and climate change)," and as such each RU also includes the Hydrologic Units and elevational limits to capture topography. The eight RUs provide for recovery within the Mogollon Rim and southern groups of populations, but also allow for recovery within smaller geographic areas such as watersheds and mountain ranges that likely also exhibit local adaptation. Although our aim is to conserve genetic diversity within and among the RUs, it is not a criterion for recovery. If all populations are lost within an RU, frogs may be imported from an adjacent RU under specific protocols found in Appendix D of the Chiricahua Leopard Frog Recovery Plan (USFWS 2007). Overall, the RUs are designed to contain areas with similar recovery challenges and to promote local conservation efforts. More detailed information is available in the recovery plan.

Although one of the most aquatic of southwestern leopard frogs (Degenhardt *et al.* 1996), Chiricahua leopard frogs are known to move among aquatic sites, and such movements are crucial for conserving metapopulations. A metapopulation is a set of local populations (subpopulations) that interact via individuals moving between local populations (Hanski and Gilpin 1991). If local populations are extirpated through drought, disease, or other factors, the populations can be recolonized via dispersal from adjacent populations. Hence, the long-term viability of metapopulations may be enhanced over that of isolated populations, even though local populations experience periodic extirpations.

The population structure of the Chiricahua leopard frog involves both metapopulations and isolated populations, as mentioned in the recovery criteria. For this species, a metapopulation should consist of at least four local populations that exhibit regular recruitment, three of which are continually in existence. Local populations should be arranged in geographical space in such a way that no local population will be greater than 5.0 miles (mi) (8.0 kilometers [km]) from at least one other local population during some part of the year unless facilitated dispersal is planned (USFWS 2007). Metapopulations should include at least one large, healthy subpopulation (e.g., at least 100 adults) in order to achieve an acceptable level of viability as a larger unit. If aquatic habitats can be managed for persistence through drought periods (e.g., supplying water via a pipeline or a well, lining a pond), overall metapopulation viability may be achievable with a smaller number of individuals per subpopulation (e.g., 40 – 50 adults) (USFWS 2007).

Isolated breeding populations are also needed as a buffer against disease because disease organisms can spread rapidly through a metapopulation as infected individuals move among aquatic sites. Chiricahua leopard frogs are reasonably likely to disperse 1.0 mi (1.6 km) overland, 3.0 mi (4.8 km) along ephemeral or intermittent drainages (water existing only briefly), and 5.0 mi (8.0 km) along perennial water courses (water present at all times of the year), or some combination thereof not to exceed 5.0 mi (8.0 km). An isolated, but robust breeding population should be located at a distance farther than 5 mi

(8 km) from other Chiricahua leopard frogs, contain at least 60 adults, and exhibit a diverse age class distribution that is relatively stable over time. A population of 40-50 adults can also be robust or strong if it resides in a drought-resistant habitat (USFWS 2007). At least two metapopulations and one isolated robust population are needed in each recovery unit to meet the recovery criteria in the recovery plan (USFWS 2007)

Discussion of how the Recovery Criteria have, or have not, been met:

Recovery Unit 1:

The requisite number of metapopulations (two) and isolated, robust populations (one) have been met (Criterion 1). Metapopulations meeting the definition in the recovery plan occur at Buenos Aires National Wildlife Refuge, Sycamore Canyon area, and Peña Blanca/Alamo Canyon. Importantly, the Refuge supports the strongest metapopulation known within the range of the species. Isolated, robust populations, as defined above, occur in the Sierrita Mountains and at Buenos Aires National Wildlife Refuge.

The appropriate protection and management of habitats for persistence of metapopulations and connectivity have not been met (Criteria 2 and 3). However, much of the Sycamore Canyon metapopulation lies within the Pajarita Wilderness area in the Coronado National Forest, and Buenos Aires National Wildlife Refuge manages for the frog.

Threats have not been eliminated (Criterion 4). American bullfrogs (*Rana catesbeiana*), crayfish (*Orconectes virilis* and possibly others), non-native fishes, illegal border activity and law enforcement response, and drought continue to threaten frogs in this RU. Chytridiomycosis, a fungal skin disease caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*), is present, but the frog is persisting with the disease, which appears to have little effect on population viability in this RU. We hypothesize that essential breeding habitats in this RU either lack stressors or include conditions that allow for persistence of Chiricahua leopard frogs with the disease (i.e., lower predation, higher population numbers, warmer waters, higher pH, and/or lower elevation).

Recovery Unit 2:

The requisite number of metapopulations (two) and isolated, robust populations (one) have not been met (Criterion 1), although we are working toward metapopulations meeting the definition in the recovery plan on the eastern slope of the Santa Rita Mountains and on the southeastern slopes of the Huachuca Mountains. An isolated, robust population occurs at Beatty's Guest Ranch in the Huachuca Mountains and is the most stable, robust population in this RU. Several other isolated populations also occur scattered across the RU, and we are currently working with partners to build a metapopulation in the Las Cienegas area.

The appropriate protection and management of habitats for persistence of two metapopulations and connectivity have not been met (Criteria 2 and 3). However, dispersal sites and corridors for connectivity have been established in the Huachuca Mountains (e.g. Ramsey Canyon), and various conservation plans and Safe Harbor Agreements have been developed or are in development in this RU.

Threats have not been eliminated (Criterion 4). American bullfrogs, crayfish, chytridiomycosis, non-native fishes, illegal border activities and law enforcement response, and wildfire continue to threaten frogs in this RU.

Recovery Unit 3:

The requisite number of metapopulations (two) and isolated, robust populations (one) have not been met (Criterion 1). A metapopulation exists in the Peloncillo Mountains; however, it lacks a robust population, and breeding is sporadic at some sites. We are working with partners to establish a metapopulation in the Chiricahua Mountains, but as yet, no Chiricahua leopard frogs occur in that mountain range. Isolated, robust populations occur in the Playas Valley, San Bernardino Valley, and at Leslie Canyon National Wildlife Refuge. Chiricahua leopard frogs likely occur in the Sierra San Luis, Sonora and Chihuahua, Mexico.

The appropriate protection and management of habitats for persistence of metapopulations and connectivity have not been met (Criteria 2 and 3). However, conservation plans and efforts provide important conservation benefits to populations in the Peloncillo Mountains tanks and Rosewood and North tanks in the San Bernardino Valley. Currently, we are working with partners in the Cave Creek/Portal area of the Chiricahua Mountains to establish frogs there.

Threats have not been eliminated (Criterion 4). Non-native fishes, chytridomycosis, prescribed fires, wildfire, illegal border activities and law enforcement response, and drought continue to threaten frogs in this RU.

Recovery Unit 4:

The requisite number of metapopulations (two) and isolated, robust populations (one) have not been met (Criterion 1). However, one metapopulation occurs in the Galiuro Mountains, and potential exists for other metapopulations in the Dragoon or Galiuro mountains. An isolated population exists in the Galiuro Mountains, but habitat is not extensive enough for it to be robust.

The appropriate protection and management of habitats for persistence of metapopulations and connectivity have not been met (Criteria 2 and 3).

Threats have not been eliminated (Criterion 4). Drought and lack of habitat continue to threaten frogs in this RU. Chytridiomycosis has not been found in recovery unit 4, but is a potential threat. Tiger salamanders (*Ambystoma mavortium*), which prey upon larval frogs, limit recovery opportunities in the Galiuro Mountains. Lack of suitable breeding habitat limits recovery opportunities in the Dragoon Mountains.

Recovery Unit 5:

The requisite number of metapopulations (two) and isolated, robust populations (one) have not been met (Criterion 1). However, if a robust population can be established in the Gentry Creek area, a metapopulation will occur there, and we are working toward establishing metapopulations in the Buckskin Hills and Ellison-Lewis creek areas. A recently established population at Moore Saddle Tank #42 near Lewis Creek has the potential to be an isolated robust population.

The appropriate protection and management of habitats for persistence of two metapopulations and connectivity have not been met (Criteria 2 and 3).

Threats have not been eliminated (Criterion 4). Bullfrogs, crayfish, non-native fishes, chytridiomycosis, wildfire, and drought continue to threaten frogs in this RU.

Recovery Unit 6:

The requisite number of metapopulations (two) and isolated, robust populations (one) have not been met (Criterion 1). Isolated, robust populations occur on Main Diamond Creek. Potential exists for a metapopulation in the Deep Creek Divide/Negrito Creek area.

The appropriate protection and management of habitats for persistence of two metapopulations and connectivity have not been met (Criteria 2 and 3).

Threats have not been eliminated (Criterion 4). Crayfish, non-native fishes, bullfrogs, wildfire, and chytridiomycosis continue to threaten frogs in this RU. Frogs in this unit are particularly sensitive to chytridiomycosis, which if present, usually results in decline and extirpation of affected frog populations. Reasons for this sensitivity in this frog population may be elucidated by an ongoing microbe study.

Recovery Unit 7:

The requisite number of metapopulations (two) and isolated, robust populations (one) have not been met (Criterion 1). Few populations are known in this unit, only one of which is robust (Rattlesnake Pasture Tank). That one robust population has potential to be part of a metapopulation, if frog populations can be established nearby.

The appropriate protection and management of habitats for persistence of two metapopulations and connectivity have not been met (Criteria 2 and 3).

Threats have not been eliminated (Criterion 4). Bullfrogs, drought, and wildfire continue to threaten frogs in this RU. Chytridiomycosis has been recently detected in the New Mexico portion of this RU.

<u>Recovery Unit 8:</u>

The requisite number of metapopulations (two) and isolated, robust populations (one) have not been met (Criterion 1). A metapopulation occurs along Seco Creek on the Ladder Ranch, and isolated populations, some of which are robust, occur at several other sites. Potential exists for establishing a metapopulation on the Mimbres River and possibly around Ash and Bolton springs east of Hurley.

The appropriate protection and management of habitats for persistence of two metapopulations and connectivity have not been met (Criteria 2 and 3). However, conservation efforts at the Mimbres River Preserve and Ladder Ranch provide important conservation benefits. Chiricahua leopard frogs from the Mimbres River have persisted with *Bd* since at least 2001, the earliest detection there. While the reasons for persistence remain unknown, conservation efforts include using these potentially *Bd*-resistant frogs for repatriation efforts and anti-chytrid skin microbe research. The Ladder Ranch conservation efforts include building a ranarium (a facility to hold, breed, rear, and head-start frogs), bullfrog removal, utilizing steel rim tanks to create refugia populations of extant populations, and maintaining water in earthen stock tanks with frogs.

Threats have not been eliminated (Criterion 4). Bullfrogs, non-native fishes, chytridiomycosis, agriculture, rural development, water diversions, and groundwater pumping continue to threaten frogs in this RU. As noted above, the Mimbres River population is persisting with *Bd*. *Batrachochytrium dendrobatidis* has not been detected within the current extent of frogs on the Ladder Ranch; however, the frog has been extirpated from surrounding drainages on the Ranch where *Bd* was detected. The Seco Creek metapopulation on the Ladder Ranch could be susceptible to declines or extirpation if *Bd* is introduced.

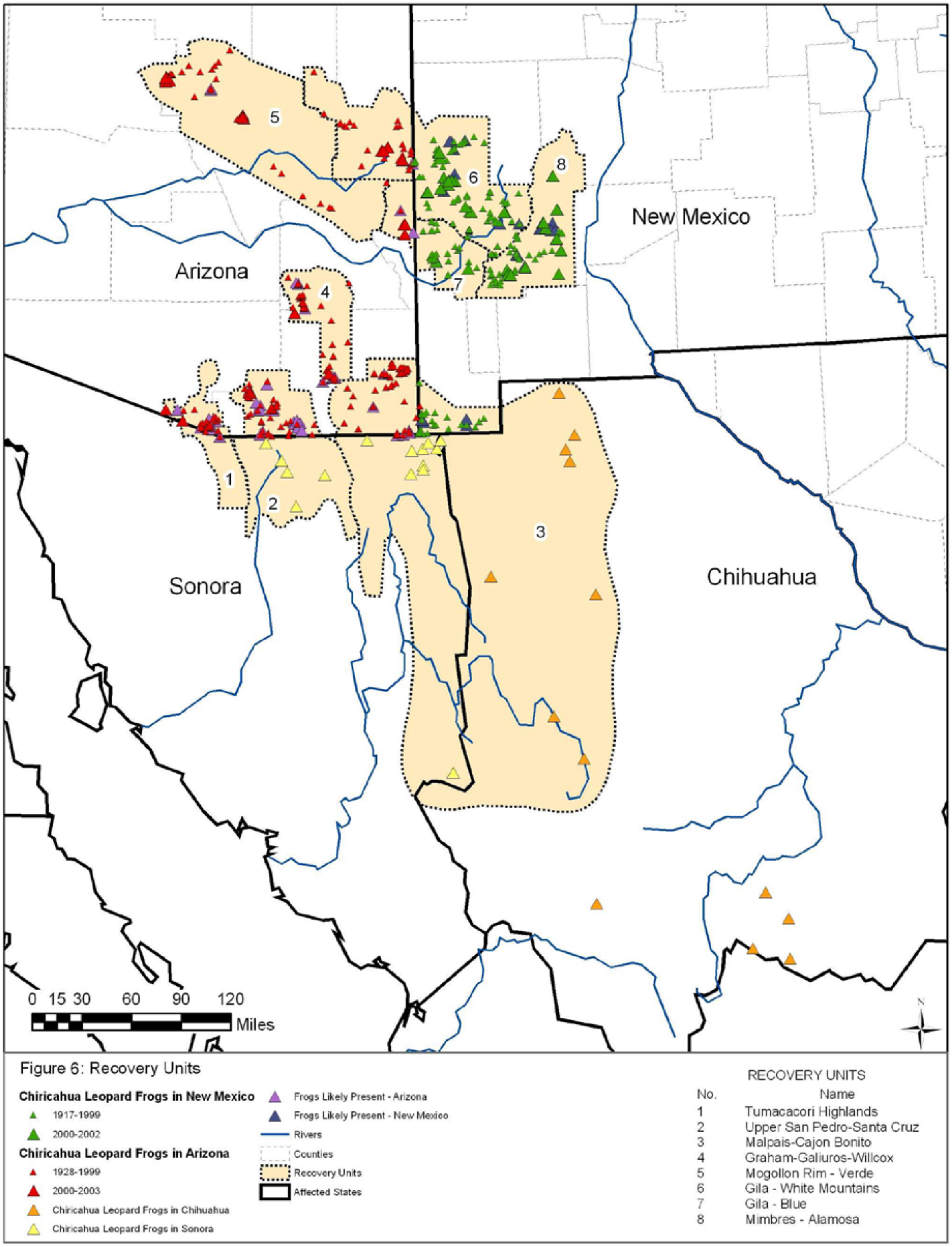

Figure 1. Map of the known range of the Chiricahua leopard frog as of 2007. The map covers areas in Arizona, New Mexico, and Mexico. All eight recovery units (RUs) are delineated.

2.3 Updated Information and Current Species Status

As background, leopard frogs (*Lithobates* [=*Rana*] *pipiens* complex), were long considered to consist of a few highly variable taxa, but are now recognized as a diverse assemblage of about 29 species (Hillis *et al.* 1983, Frost 2004, Hillis and Wilcox 2005), with more species awaiting identification (USFWS 2007). The Chiricahua leopard frog can grow up to 4.3 inches (in) (10.9 centimeters [cm]) long, and is often green with a leopard-like patterning, pale tubercles on the rear thighs, rough skin on the back and thighs, and a stocky body. The species occurs at elevations of 3,281 to 8,890 feet (ft) (1,000 to 2,710 meters [m]) in central and southeastern Arizona, west-central and southwestern New Mexico, and the sky islands and Sierra Madre Occidental of northeastern Sonora and western Chihuahua, Mexico. The distribution of the Chiricahua leopard frog in Mexico is unclear, as systematic or intensive surveys for Chiricahua leopard frogs have not been conducted (USFWS 2007).

The Chiricahua leopard frog is an inhabitant of montane and river valley cienegas, springs, pools, cattle tanks, lakes, reservoirs, streams, and rivers. The species requires permanent or semi-permanent pools for breeding and water characterized by low levels of contaminants and moderate pH. Chiricahua leopard frogs may be excluded or exhibit periodic die-offs where *Batrachochytrium dendrobatidis* (*Bd*), a pathogenic chytridiomycete fungus, is present. The diet of the Chiricahua leopard frog includes primarily invertebrates such as beetles, true bugs, and flies, but fish and snails are also eaten (Christman and Cummer 2006).

The life history of the Chiricahua leopard frog can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily aquatic (USFWS 2007). The species has a distinctive call and males can be temporarily territorial (USFWS 2007). Amplexus is axillary and the male fertilizes the eggs as the female attaches a spherical mass to submerged vegetation. Eggs are laid mainly from February into October, with most masses found in the warmer months (USFWS 2007). Numbers of eggs in a mass range from 300 to 1,485 and may be correlated with female body size (Jennings and Scott 1991, Sredl and Jennings 2005). Hatching time of egg masses in the wild ranges between 8-14 days, depending on water temperature (USFWS 2007). Upon hatching, tadpoles are mainly herbivorous and remain in the water, where they feed and grow, with growth rates faster in warmer conditions. Tadpoles have a long larval period, from three to nine months, and may overwinter. After metamorphosis, Chiricahua leopard frogs eat an array of invertebrates and small vertebrates and are generally inactive between November and February (USFWS 2007). Males reach sexual maturity at 2.1-2.2 in (5.3-5.6 cm), a size they can attain in less than a year (Sredl and Jennings 2005). Under ideal conditions, Chiricahua leopard frogs may live as long as 10 years in the wild (Platz *et al.* 1997).

Chiricahua leopard frogs can be found active both day and night, but adults tend to be active more at night than juveniles (Sredl and Jennings 2005). Chiricahua leopard frogs presumably experience very high mortality (greater than 90 percent) in the egg and early tadpole stages, high mortality when the tadpole turns into a juvenile frog, and then relatively low mortality when the frogs are adults (Zug *et al.* 2001, USFWS 2007). Adult and juvenile Chiricahua leopard frogs avoid predation by hopping to water (Frost and Bagnara 1977). They also possess an unusual ability among members of the *Rana pipiens* complex; they can darken their ventral skin under conditions of low reflectance and low temperature (Fernandez and Bagnara 1991; Fernandez and Bagnara 1993), a trait believed to enhance camouflage and escape predation (USFWS 2007).

Males have larger home range sizes than females, with the largest home range for a male documented at 251,769 ft^2 (7,674 by 32 ft, or 23,390.2 m^2 [2,339 by 9.8 m]) (USFWS 2007). The maximum distance moved by a radio-telemetered Chiricahua leopard frog in New Mexico was 2.2 miles (3.5 km) in one direction (preliminary findings of telemetry study by R. Jennings and C. Painter, Technical Subgroup, 2004). Although amphibians are known to have limited dispersal and colonization abilities due to physiological constraints, limited movements, and high site fidelity (Blaustein *et al.* 1994), Chiricahua leopard frogs can disperse to avoid competition, predation, or unfavorable conditions (Stebbins and Cohen 1995). Dispersal most likely occurs within favorable habitat, making the maintenance of corridors that connect disjunct populations possibly critical to preserving populations of frogs. Active or passive dispersal (while carried along streamcourses) of juveniles or adults to discrete aquatic habitats facilitates the creation and maintenance of metapopulations (USFWS 2007), an important option for a water-dependent frog in an unpredictable environment like the arid Southwest.

For far more detailed information on this species, please refer to the following, which is the baseline in regard to the current status, biology, and threats to the Chiricahua leopard frog:
U.S. Fish and Wildlife Service. 2007. Chiricahua Leopard Frog (*Rana chiricahuensis*) Recovery Plan. Region 2, U.S. Fish and Wildlife Service, Albuquerque, NM. 429 pp.

2.3.1 Biology and Habitat

2.3.1.1 New information on the species' biology and life history

Since the 2007 publication of the recovery plan, there has been no new information concerning the biology or life history of the Chiricahua leopard frog.

2.3.1.2 Trends in population, demography, and spatial distribution

<u>Arizona</u>

A review of the status of the species in Arizona from 2002, when the species was listed, to 2009 was conducted by Rorabaugh (2010). A comparison of survey results during 2005-2009 versus 1999-2002 revealed increasing numbers of sites occupied by frogs from 2002-2008. The total number of occupied sites increased from 49 in 2002 to 80 in 2008 and 90 in 2009, while the number of robust breeding populations increased from 5 in 2002 to 13 in 2008, and then declined slightly to 11 in 2009. The total number of breeding populations increased from 26 in 2002 to 34 in 2008 and then declined by 1 to 33 in 2009. These trends were also generally reflected at the recovery-unit level of analysis. Exceptions included a reduction in number of breeding populations in recovery unit 3 from three to two and in recovery unit 6 from three to zero. Recovery unit 5 exhibited a reduction in the number of robust breeding populations from two to zero (See Figure 1).

Although the data suggest substantial gains, basing status and trends on differences in numbers of occupied sites from 2002-2009 can be problematic for several reasons. First, if increasing trends are real, they may represent population response to temporarily favorable environmental conditions, such as adequate summer rains that allow dispersal, rather than an intrinsic improvement that will endure over time. Second, there are sources of bias that affect the conclusions. For instance, both data sets likely underestimate the number of occupied sites existing at the time, because some sites were unknown or surveys had not been conducted within the last three years to categorize all sites as occupied or unoccupied. But there is further bias in the survey data in that the 2009 data set benefits from recent discoveries of populations that could have existed in 2002, but we did not know of them at the time.

The latter type of bias can be eliminated by adding to the 2002 total all of the occupied sites that were discovered after 2002, except for those for which we are reasonably certain were unoccupied in 2002. If analyzed in this way, the total number of occupied sites, in 2002, increases from 49 to 83. This is roughly the same number of occupied sites as in 2008 (85). Based on this, the total number of occupied sites was fairly stable or increasing slightly in Arizona from 2002 (83) to 2008 (85) and 2009 (92). However, this correction inserts yet another type of bias into the sample – analyzed in this way, the 2002 total is based not only on what was found during 1999 to 2002, but also surveys during period 2003 to 2009. Yet the 2008 and 2009 totals are only based on surveys during 2005-2008 and 2006-2009 respectively. Number of occupied sites in 2009 would no doubt increase if we could add in new sites during the equivalent future period (through 2016).

As a result, concluding there were 83 extant sites in 2002, 85 in 2008, and 92 in 2009, is likely the worst case scenario, in that this analysis is most likely to show any declines, if they occurred from 2002-2009. The actual trend is probably somewhere between that (roughly stable) to what was concluded in the previous analysis (substantial increases). In conclusion, there is no evidence of decline in Arizona, rather, the data suggest at least modest increases.

New Mexico

A similar analysis for New Mexico populations was not possible because not all sites have been monitored annually and much of the reported survey information is reported as presence or absence. Due to the evolving nature of Chiricahua leopard frog monitoring since the early 1990s and the ability of frogs to move up to 5 miles (8 km), surveys have had different definitions of sites and populations over time. Often site boundaries are indistinct making some connected areas a single site, and other connected areas several sites. Thus it is difficult to assess the frog's status by enumerating sites and often comparisons among sites are not equivalent. However, there are sufficient data available to conclude that the declining status of the frog in New Mexico pre-listing has continued to decline annually since listing.

As background, the final rule listing the species indicated the frog had been found at 41 sites from 1994-1999, and 31 of these 41 sites were verified as extant during 1998-1999. The rule explains that frogs were found at only 8 of 34 surveyed sites (of the original 41 sites) in 2000. The recovery plan indicated that 30-35 populations of Chiricahua leopard frogs were likely extant in New Mexico at the time of writing (2006-7). The tally of these 30-35 populations included dispersal sites, which indicates that not all of these populations were robust, breeding sites. Starting with the 41 sites from 1994-1999, 27 of those sites are now extirpated, 4 of them are considered unstable with low population numbers or are possibly extirpated, 2 are considered dispersal observations with no reproduction, 1 has an unknown status due to inaccessibility, and 7 sites support reproduction and no significant die-off or population loss has been observed. Based on these data, 27 of the 41 sites are considered extirpated, representing a 66 percent drop in the known Chiricahua leopard frog sites in New Mexico during this 5-year period.

Since listing in 2002, an additional 30 new sites have been identified. To date, of these 30 new sites, 15 have become extirpated, 6 are unstable with low population numbers or are possibly extirpated, 4 are considered dispersal observations with no reproduction, 1 site is on private property with an unknown population status, and at 4 sites reproduction is occurring and no significant die-off or population loss has been observed. New sites have been found due to increased surveying efforts in remote areas and growing access to private lands through partnership activities. Although undiscovered occupied sites may still exist, the rate and likelihood of finding new sites will diminish, as the area of unsurveyed habitat is reduced each year. Furthermore, while the frog has a large capacity for dispersal, because of the locality of the new observations, we assume that most of the new observations were existing locations and not newly colonized locations. Thus in the past eight years, these newer sites have reflected a similar trend of decline, with half of the sites disappearing.

In summary, based on recent monitoring of Chiricahua leopard frog populations from 1994 to 2010, a total of 71 occupied sites has been counted. Since 1994, at least 42 of these sites have been extirpated, accounting for 59 percent of the known sites in a 16-year period. At present, we are aware of 11 extant reproductive sites (1 of which is a robust metapopulation, and 2 of which are on private land and have not been monitored in recent years but thought to be extant); 10 sites with low population numbers or possible extirpation; 6 dispersal areas; and 2 sites where the status is unknown. These numbers include dispersal sites, most often comprised of a single juvenile frog, with no observed reproduction. While the current overall number of extant sites (29) does not differ greatly from that reported in the 2007 final recovery plan (30-35), comparing these site numbers is misleading. Specifically, there are two reasons for this. The first is due to the inconsistent definition of a site or a population in different monitoring schemes, prohibiting a direct comparison of data within or among sites. For example, the metapopulation on the Ladder Ranch is currently known as the Seco Creek complex, consisting of seven reproductive sites located in close proximity, yet it is currently counted as one site. It is not known how this complex was quantified in the final listing rule or the in the recovery plan. Likewise, there are currently three sites counted in the Mimbres River; however, all are in the dispersal distance of the others, with two different landowner situations. This is counted as three separate sites at this time but may be transformed into one site in the near future as we refine our definitions of boundaries and metapopulations. The second reason is because the apparent high turnover rate of frog populations does not represent natural population dynamics, but instead reflects actual extinctions without the recolonizations that would be found in a functioning metapopulation. Thus the recent observations of new sites mask continued population losses of the Chiricahua leopard frog in New Mexico.

Disease, particularly infection caused by *Bd*, has accounted for the majority of Chiricahua leopard frog declines. This disease seems to present more of a threat the frog in New Mexico than it does in Arizona, perhaps due to the higher elevations and cooler conditions found at sites in New Mexico. However, non-native species (bullfrogs, crayfish, and non-native fish) also continue to significantly impact extant populations and threaten the frog in New Mexico. All remaining frog populations in New Mexico are extremely vulnerable to extirpation from disease, non-native species, small population sizes, habitat drying, and lack of connectivity between other suitable habitats or populations.

In recent years, New Mexico Chiricahua leopard frog partners have gained momentum in conservation actions. In an effort to stave off permanent genetic losses, much of the recovery activities in New Mexico have been focused on creating off-site refugia populations. This entails collecting wild eggs, tadpoles, or metamorphs and bringing them into captivity for rearing and disease testing and treatment if needed, and releasing them into confined steel rim tanks. Currently, the NMESFO and the Bureau of Land Management have the capacity to rear, hold, and treat animals; the Forest Service has set up a quarantine holding facility (for first use in Spring 2011); and the Ladder Ranch has outdoor holding pens for adult frogs (for captive reproduction). For the Chiricahua leopard frog in New Mexico, our intent is that not only will the refugia sites serve as a back-up if there is a die-off at the source population, but that with time, they will also serve as a source for additional repatriation efforts. The facilities that are contributing to these efforts will also serve to produce animals for repatriation projects once extant populations have been boosted. As of 2010, we have attempted to establish eight refugia populations. Two of the source populations for the eight refugia sites have since experienced die-off and are extirpated.

Mexico: Sonora and Chihuahua

Based on published and unpublished reports and perusal of Sonora, Mexico, collection data from 23 museums, the Chiricahua leopard frog is known from about 26 localities in Chihuahua, Mexico (Lemos-Espinal and Smith 2007) and 19 localities in Sonora. "*Lithobates* [*Rana*] *chiricahuensis*" have been reported as far south as the Mexican state of Aguascalientes, but frogs south of central Chihuahua are of questionable identification (USFWS 2007). Based on limited surveys, populations of leopard frogs, gartersnakes, and other native aquatic herpetofauna are generally more intact and non-native predators are much less widely distributed in Sonora and at least parts of Chihuahua (Rosen and Melendez 2010, Lemos-Espinal and Smith 2007, Rorabaugh 2008). However, specifically for the Chiricahua leopard frog, data are insufficient to determine status or trends in Mexico. None of the Chiricahua leopard frog localities in Sonora have been revisited recently, with the exception of one in the Sierra Los Ajos. No frogs were found at that site (L. Portillo, pers. comm. 2009). Chiricahua leopard frogs have been observed recently at several sites in Chihuahua (R. Jennings, pers. comm. 2007), but not enough is known to assess status or trends.

In conclusion, the data suggest the status of the Chiricahua leopard frog is at least stable and probably improving in Arizona, declining in New Mexico, and unknown in Mexico. In pooled data for the United States, a worst case analysis shows essentially no change in the number of occupied sites from 2002 to 2009 (133 versus 131, respectively); however, as discussed above, this likely underestimates the status of the species in Arizona, overestimates the status of the species in New Mexico, and includes data that are not standardized to be truly comparable. The actual situation is probably that the status of the species is stable in the U.S overall, but the different conditions between Arizona and New Mexico indicate that improvement is occurring only in Arizona at this time, while in New Mexico, frog numbers continue to decline. Continued and new aggressive recovery actions are needed to address threats to the species rangewide, to maintain positive trends in Arizona, to stabilize population losses in New Mexico, and to assist partners in Mexico with their conservation efforts. If on-going recovery actions are interrupted, drought worsens, or other threats intensify, the status of the species could easily deteriorate.

2.3.1.3 Genetics, phylogeny, and taxonomy

As noted in section 1.2 (Methodology used to complete the review), when the Chiricahua leopard frog was listed as a threatened species on June 13, 2002 (67 FR 40790), we recognized the scientific name as *Rana chiricahuensis*. Since that time, the genus name *Lithobates* was proposed by Frost *et al.* (2006) and adopted by the Society for the Study of Amphibians and Reptiles in their most recent listing of scientific and standard English names of North American amphibians and reptiles north of Mexico (Crother 2008). We recommend to accept the new scientific name of the Chiricahua leopard frog as *Lithobates chiricahuensis.*

Northern populations of the Chiricahua leopard frog in the Mogollon Rim region of east-central Arizona to the eastern bajada of the Black Range in New Mexico are disjunct from populations to the south. Previous studies revealed some genetic differentiation from frogs in southeastern Arizona (Platz and Grudzien 1999, Goldberg *et al.* 2004), suggesting the northern form could represent a distinct taxon. If the species was split, the two resulting taxa would be more imperiled than the currently recognized Chiricahua leopard frog because fewer populations would be represented in each new taxon, and threats are widespread and affecting populations in both the Mogollon Rim and southern populations. Goldberg *et al.* (2004) found a 2.4 percent mean difference in mitochondrial DNA (mtDNA) sequences between these two lineages, which the authors point out is less than among populations of *Rana cascadae* in Washington, Oregon, and California (3.2-4 percent, Monsen and Blouin 2003) and subspecies of *R. porosa* (3.7 percent, Sumida *et al.* 2000). It is also less than the average sequence divergence between eastern and western haplotypes of *Lithobates pipiens* (3 percent, Hoffman and Blouin 2004). However, sequence divergence between populations of *L. yavapaiensis* and *L. onca* range from only 1.70-2.39 percent (Jaeger *et al.* 2001). Goldberg *et al.* (2004) concluded that the Mogollon Rim and southern lineages of the Chiricahua leopard frog could represent two distinct species, but examination of behavioral, ecological, and morphological differences between the groups was needed before this determination could be made. These studies have not yet been conducted.

More recently, Hillis and Wilcox (2005) demonstrated some differences in rDNA sequences between Chiricahua leopard frogs from the Mogollon Rim in Arizona and Durango, Mexico. The Durango specimens could represent a southern population of the Chiricahua leopard frog, but could also be the recently described *L. lemosespinali*, which is very similar to the Chiricahua leopard frog, but has not been documented that far south (Smith and Chiszar 2003). To further complicate matters, Lemos-Espinal and Smith (2007) reported that Chiricahua leopard frogs from Chihuahua, Mexico, are much smaller (maximum snout-vent length of 58 mm) than in the United States (maximum snout-vent length of 121 mm). Together, this information suggests additional cladistic structure may exist within what is now considered *L. chiricahuensis*.

Goldberg *et al.* (2004) also examined the relationships between the Ramsey Canyon leopard frog (*L. subaquavocalis*) and the Chiricahua leopard frog. The Ramsey Canyon leopard frog is found on the eastern slopes of the Huachuca Mountains in Cochise County, Arizona, located entirely within the Chiricahua leopard frog's range. They found *subaquavocalis* to be on a short branch within the southern Arizona clade of *chiricahuensis* and suggested the two were conspecific. This recommendation was adopted by the Society for the Study of Amphibians and Reptiles (Crother 2008). Therefore, we support this finding from the scientific literature and recommend that the Ramsey Canyon leopard frog (*L. subaquavocalis*) no longer be considered a distinct species, but instead synonymous with the Chiricahua leopard frog (*L. chircauensis*).

The phylogeny and structure within *Lithobates chiricahuensis* was recently examined by USGS scientist Melanie Culver and her colleagues at the University of Arizona (Herrmann *et al.* 2009). They conducted both mtDNA and microsatellite analysis using 347 samples from throughout the range of the Chiricahua leopard frog. As of this writing, we only have a preliminary report, but the study is quite revealing as to structuring within *chiricahuensis.* The Bayesian derived phylogenetic tree and the Median-joining network from mtDNA sequences indicate four major groupings within the Chiricahua leopard frog. A Mogollon Rim population found on the Coconino and Apache-Sitgreaves National Forests in Arizona is the most basal or ancestral and has the highest genetic diversity. The other three groupings or populations are: 1) a widespread derived clade found throughout the range of the species, 2) a derived clade that occurs from Hidalgo County, New Mexico, south through the Sierra Madre of Mexico, and 3) a basal clade along the Mogollon Rim of Arizona and into the Gila National Forest in New Mexico. Signatures of most of these groupings can be found throughout the range of the frog, suggesting considerable mixing and gene flow. The widespread clade, found throughout the range of the species, may be indicative of waves of migrations that were very successful overall, but have not swamped out the historical signal on the Mogollon Rim.

Herrmann *et al.* (2009) conducted another analysis of the microsatellite data at a finer resolution using a Bayesian program GENELAND to identify the maximal number of groups. In that analysis, 9 to 16 groups were indicated, although the support for 16 groups was weak. These groups were found to be localized with no widespread groups identified.

In conclusion, the authors found no evidence for more than one taxon within *L. chiricahuensis*, although they admit that some outliers in the samples need further investigation. Herrmann *et al.* (2009) did not include in their study any genetic samples from south of west-central Chihuahua. A genetic investigation of additional samples from Mexico would be insightful and may help clarify relationships with *Lithobates lemosespinali* and *L. montezumae.*

Hillis and Wilcox (2005) noted morphological similarities between the extinct Vegas Valley leopard frog (*Lithobates fisheri*) from southern Nevada and Mogollon Rim populations of Chiricahua leopard frogs. They suggested these northern populations of Chiricahua leopard frogs may be referred to as "*Rana*" (*Lithobates*) *fisheri*, despite the fact that *L. fisheri* in the Vegas Valley was disjunct from Mogollon Rim *chiricahuensis* populations by about 230 miles. However, work is underway to examine the molecular genetics of *L. fisheri* specimens preserved in ethanol. This work could find the two currently recognized species to be conspecific, in which case the scientific name of the Chiricahua leopard frog would revert to *L. fisheri*, and the historical range of the species would extend to the Vegas Valley in Nevada.

2.3.1.4 Habitat or ecosystem conditions

Adult and juvenile Chiricahua leopard frogs need aquatic breeding and overwintering sites, as well as corridors for dispersal, in the context of metapopulations and as isolated populations. Aquatic breeding habitat is essential for providing space, food, and cover necessary to sustain all life stages of Chiricahua leopard frogs. It consists of permanent or nearly permanent aquatic habitats from about 3200-8,900 ft (975-2,715 m) elevation with deep (greater than 20 in (0.5 m)) pools in which nonnative predators are absent or occur at such low densities and in complex habitats to allow persistence of Chiricahua leopard frogs (USFWS 2007). Included are cienegas or springs, pools, livestock tanks, lakes, reservoirs, streams, and rivers. Sites as small as 6.0 ft (1.8 m) diameter steel troughs can serve as important breeding sites, particularly if that population is part of a metapopulation that can be recolonized from adjacent sites if extirpation occurs. Some of the most robust extant breeding populations are in dirt livestock tanks. Habitats for Chiricahua leopard frogs are found on Federal, state, Tribal, local, and privately owned lands.

To be considered essential breeding habitat, water must be permanent enough to support breeding, tadpole development to metamorphosis (change into a frog), and survival of frogs. Juvenile and adult frogs need moisture for survival, including sites for hibernation. Overwintering sites of Chiricahua leopard frogs have not been investigated; however, hibernacula (shelter occupied during winter by non-active animals) of related species include sites at the bottom of well-oxygenated ponds, burial in mud, or moist caves (USFWS 2007). Given these requirements, sites that dry out for one month or more will not provide essential breeding or overwintering habitat. However, occasional drying for short periods (less than one month) may be beneficial in that the frogs can survive, but nonnative predators, particularly fish, and in some cases, American bullfrogs and populations of aquatic forms of tiger salamanders, will be eliminated during the dry period (USFWS 2007). In occupied essential breeding habitat, if nonnative crayfish, predatory fishes, bullfrogs, or barred tiger salamanders are present, these predators occur only as rare dispersing individuals that do not breed, or are at low enough densities in habitats that are complex and with abundant escape cover so that both Chiricahua leopard frogs and nonnative species can persist (Sredl and Howland 1994, USFWS 2007, Witte *et al.* 2008).

Water quality requirements include sites where pH is equal to or greater than 5.6 (Watkins-Colwell and Watkins-Colwell 1998), salinities are less than 5 parts per thousand (Ruibal 1959), and chemical pollutants, including but not limited to heavy metals, pesticides, mine runoff, and fire retardants, do not exceed the tolerance of Chiricahua leopard frogs (Rathbun 1969, U.S. Bureau of Land Management 1998, Boone and Bridges 2003, Calfee and Little 2003, Sparling 2003, Relyea 2004, USFWS 2007, Little and Calfee 2008). White (2004) provides specific pesticide use guidelines for minimizing impacts to the Chiricahua leopard frog.

Essential aquatic breeding sites require some open water and, ideally, vegetative cover with some bare substrate. Chiricahua leopard frogs can be eliminated from sites that become entirely overgrown with cattails (*Typha* sp.) or other emergent plants. At the same time, frogs need some emergent or submerged vegetation, root masses, undercut banks, fractured rock substrates, or some combination thereof as refugia from predators and extreme climatic conditions (Sredl and Jennings 2005). Uplands adjacent to water provide essential foraging and basking sites, particularly in riparian vegetation and on open banklines out to the edge of riparian vegetation. Vegetation in these areas provide habitat for prey species and protection from terrestrial predators (those living on dry land). In particular, Chiricahua leopard frogs use these upland areas during the summer rainy season.

Dispersal habitat provides routes for connectivity and gene flow among local populations within a metapopulation, which enhances the likelihood of metapopulation persistence and allows for recolonization of sites that are lost due to drought, disease, or other factors (Hanski and Gilpin 1991, USFWS 2007). The most likely dispersal routes may include combinations of ephemeral, intermittent, and perennial drainages, as well as uplands. Some vegetation cover for protection from predators, and aquatic sites that can serve as buffers against desiccation (drying) and stop overs for foraging (feeding), are desirable along dispersal routes. A lack of barriers that would block dispersal is critical. Features on the landscape likely to serve as partial or complete barriers to dispersal include cliff faces and urban areas (USFWS 2007), reservoirs 20 acres (ac) (50 hectares (ha)) or more in size that are stocked with sportfishes or other nonnative predators, highways, major dams, walls, or other structures that physically block movement (Todd and Andrews 2008, Eigenbrod *et al*. 2009, 75 FR 12818). The effects of highways on frog dispersal can be mitigated with frog fencing and culverts (USFWS 2007). Unlike some other species of leopard frogs, Chiricahua leopard frogs have only rarely been found in association with agricultural fields; hence, agriculture may also serve as a barrier to movement. Detailed studies of dispersal and metapopulation dynamics of Chiricahua leopard frogs have not been conducted; however, Jennings and Scott (1991) noted that maintenance of corridors used by dispersing juveniles and adults that connect separate populations may be critical to conserving populations of frogs.

The habitats occupied by Chiricahua leopard frogs typically contain introduced predators (American bullfrogs, crayfish, fishes, and tiger salamanders) and the introduced fungal skin disease, chytridiomycosis. These non-native predators are the primary predators of Chiricahua leopard frogs in all stages of the frog's life cycle (listing factors A and C), and chytridiomycosis is a proximal cause of amphibian decline around the globe (listing factor C). Absence of chytridiomycosis is crucial for population persistence in some regions, particularly in west-central New Mexico. However, some populations persist with the disease (e.g., sites between Interstate 19 and the Baboquivari Mountains, Arizona) with few noticeable effects on demographics or survivorship. Persistence with disease is enhanced in warm springs and at lower elevations with warmer water (USFWS 2007). Both of these threats are serious and very difficult to ameliorate; however, the recovery plan presents several options for management as well as research into techniques to address these recovery challenges.

To address habitat threats to the frog, the Southwest Endangered Species Act Team (2008) published "Chiricahua leopard frog (*Lithobates* [*Rana*] *chiricahuensis*) considerations for making effects determinations and recommendations for reducing and avoiding adverse effects." This document includes detailed descriptions of how fire management, construction, native fish recovery, and livestock management projects may affect the frog and its habitat.

Some new information affecting the Chiricahua leopard frog's habitat was included in a report was prepared by staff of the U.S. Geological Survey's Columbia Environmental Research Center in Columbia, Missouri (Little and Calfee 2008) summarizing studies of the effects of various pesticides, metals, and piscicides on Chiricahua leopard frog tadpoles. Pesticides were tested with and without ultra-violet radiation. The study found that Chiricahua leopard frog tadpoles are unlikely to be affected in the field by the herbicides tested. Cadmium and copper were toxic at levels less than those observed at some field locations, and because of toxicity of rotenone, the authors suggested using this piscicide when tadpoles are not present.

Witte *et al.* (2008) examined factors associated with disappearance of Chiricahua leopard frog populations. The only habitat-based factor that was important was elevation. Populations were more likely to be extirpated at higher elevations. Based on studies of other amphibian species elsewhere in the world, the authors suggested global warming and other climate changes may challenge ranids and/or favor pathogens at higher elevations. But because Chiricahua leopard frogs tend to persist more frequently with the disease at lower, warmer sites (USFWS 2007), the tendency for high elevation sites to be colder than those at lower elevations may be more important in explaining the disappearance of Chiricahua leopard frog populations than predicted changes in climate.

Ongoing recovery actions, including reestablishment of populations, are focusing on sites with permanent or nearly permanent water, sometimes where water levels can be managed (e.g. wells and pumps, or water pipelines). The recovery plan recommends measures to increase the permanency of water at sites subject to drought and drying. So although climate models predict continuing drought, such drought can be managed in some cases (at least in small aquatic systems).

2.3.4. Conservation Measures: An Aggressive Recovery Program

<u>Habitat preservation, enhancement; frog reintroductions</u>: State, private, Federal partners

Since before the species was listed, state, Federal, and private partners have been engaged in conservation and recovery work for the Chiricahua leopard frog. The first population reestablishments occurred in 1995 in Ramsey Canyon in southeastern Arizona, and recently the 10,000th Chiricahua leopard frog produced by the Phoenix Zoo was released into recovery unit 5. Since population reestablishments have begun, the frog has been introduced at 30 sites, of which frogs are still persisting at 23. Refugia have been established at 15 sites; they are still present at 11 of those. Furthermore, many existing populations have been augmented with headstarted tadpoles or metamorphic frogs, and in some cases wild-to-wild translocations of egg masses. Non-native predator control in the Altar Valley and in the Peña Blanca regions of recovery unit 1 have allowed Chiricahua leopard frogs to recolonize, on their own, 13 or more sites. Elimination of bullfrogs and other non-native species have allowed persistence at a number of other sites. Habitat has been created or enhanced at numerous sites in all recovery units. These efforts have been most prevalent in Arizona, where the Arizona Game and Fish Department has had two or more employees dedicated to ranid frog conservation since before the species was listed, and numerous public and private partners exist.

State and private lands conservation: Arizona Game and Fish Department - Safe Harbor Agreement

A statewide safe harbor agreement exists in Arizona. Arizona Game and Fish Department is the permit holder, and they enroll landowners under certificates of inclusion. A similar, umbrella safe harbor agreement exists with the Malpai Borderlands Group in southeastern Cochise County, Arizona, and southwestern Hidalgo County, New Mexico. Another safe harbor agreement exists with the Barboot/99 Bar ranches, Cochise County, Arizona, and Malpai Borderlands Group has a habitat conservation plan that addresses the frog. Conservation stewardship on private lands such as at the Ladder Ranch, Sierra County, New Mexico; The Nature Conservancy properties on the Mimbres River, Grant County, New Mexico, and Ramsey Canyon, Cochise County, Arizona; and Beatty's Guest Ranch and the Southwest Research Station, Cochise County, Arizona, all contribute to recovery of the Chiricahua leopard frog. The strongest metapopulation of Chiricahua leopard frogs exists on the Buenos Aires National Wildlife Refuge, Pima County, Arizona, which actively manages for this species. Another crucial recovery site is on the Leslie Canyon National Wildlife Refuge, Cochise County, Arizona. The Forest Service is an especially important partner, as most of the historical and current populations occur on or near Forest Service lands. The Tonto National Forest, in particular, has worked diligently to recover the Chiricahua leopard frog on the Pleasant Valley and Payson Ranger districts.

Habitat improvement, captive rearing: Partners for Fish and Wildlife, Bureau of Reclamation, National Fish and Wildlife Foundation

The USFWS has been aggressive at using Partners for Fish and Wildlife Service funds to improve habitats, control non-native predators, and construct rearing and captive reproduction facilities for the frog. In late fiscal year 2010, $100,000 of Bureau of Reclamation money was obligated for Chiricahua leopard frog recovery projects in accordance with a biological opinion for their Central Arizona Project. Projects to be funded include rearing facilities at the Arizona-Sonora Desert Museum, the Fish and Wildlife Service's Ecological Service's Office in Albuquerque, and Ladder Ranch; materials and services for disease testing and prevention; purchase of steel tanks as refugia; and habitat improvements. In 2009, the National Fish and Wildlife Foundation began funding their Sky Islands Grasslands Initiative. This is a multi-year, multi-million dollar funding initiative to conserve grasslands and associated imperiled species in southeastern Arizona, southwestern New Mexico, and adjacent portions of Mexico. Two projects were funded in 2009 that target conservation of Chiricahua leopard frogs and other aquatic species.

Collaborative Research: US Geological Survey (USGS) and University of Arizona, National Wildlife Health Center

The research project "Efficacy of using a Bacterial Microbe as a Strategy for Resisting *Batrachochytrium dendrobatidis* infection in the Chiricahua leopard frog (*Rana chiricahuensis*)" was funded in 2009. The principle investigators are Michael J. Adams, (PI), Research Ecologist, USGS Forest & Rangeland Ecosystem Science Center, Corvallis, Oregon; Cecil Schwalbe, Ph.D. (Co-PI), Ecologist and Assistant Professor, USGS SBSC Sonoran Desert Research Station, University of Arizona, Tucson; and David E. Green, DVM, (Co-PI) Veterinary Pathologist, National Wildlife Health Center, Madison, Wisconsin. It is hoped that this 'probiotics' study may lead to a treatment for chytridiomycosis that would be effective for wild populations of frogs.

Recovery Team for the Chiricahua Leopard Frog

The recovery program is coordinated by a two-tier recovery team, including regional steering committees and local recovery groups. Three steering committees oversee recovery in west-central New Mexico, southeastern Arizona and southwestern New Mexico, and on the Mogollon Rim, Arizona. Numerous local recovery groups implement recovery actions on the ground in specific areas. Steering committees and local recovery groups consist of both technical and stakeholder representatives. The steering committees meet annually, and local recovery groups meet as needed to facilitate recovery actions. These groups report their progress, which is synthesized into annual recovery updates for each of the three steering committee regions. These updates are posted on the Chiricahua leopard frog recovery website (http://www.fws.gov/southwest/es/arizona/CLF_Recovery_Home.htm).

2.3.2 Five-factor Analysis

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

The recovery plan lists the following threats to habitat or range of the Chiricahua leopard frog: contaminants; mining, including mining-related contaminants; dams; diversions; stream channelization; groundwater pumping; woodcutting; urban and agricultural development; road construction; grazing by livestock and elk; climate change; and altered fire regimes. Habitat threats that remain important today are degradation and loss of habitat as a result of drought, water diversions and groundwater pumping; livestock management that degrades frog habitat; a history of fire suppression and grazing that has increased the likelihood of crown fires; mining; development; environmental contamination; disruption of metapopulation dynamics via physical blockage of dispersal corridors; and the dynamic nature of frog habitats. Although these threats are widespread and varied, a threats assessment that was accomplished as part of the recovery plan showed predation by non-native species (Section 2.3.2.3) and chytridiomycosis (Section 2.3.2.3) as consistently more important threats than these habitat-based factors (USFWS 2007)

Chiricahua leopard frogs are fairly tolerant of variations in water quality, but likely do not persist in waters severely polluted with cattle feces (USFWS 2007), or runoff from mine tailings or leach ponds (Rathbun 1969, U.S. Bureau of Land Management 1998, USFWS 2007). Furthermore, variation in pH, ultraviolet radiation, and temperature, as well as predation stress can alter the potency of chemical effects (Akins and Wofford 1999, Monson *et al.* 1999, Reylea 2004). Chemicals may also serve as a stressor that makes frogs more susceptible to disease, such as chytridiomycosis (Parris and Baud 2004). The effects of pesticides and other chemicals on amphibians can be complex because of indirect effects on the amphibian environment, direct lethal and sub-lethal effects on individuals, and interactions between contaminants and other factors associated with amphibian decline (Sparling 2003, Reylea 2008).

A copper mine (the Rosemont Mine) has been proposed in the northeastern portion of the Santa Rita Mountains, Pima County, Arizona (recovery unit 2), the footprint of which includes several sites recently occupied by Chiricahua leopard frogs. Recent research (Section 2.3.2) indicates that Chiricahua leopard frog tadpoles are sensitive to cadmium and copper above certain levels (Little and Calfee 2008), making the introduction of copper into Chiricahua leopard frog habitat a possible significant threat. No analyses have been conducted yet to quantify how the frogs and their habitats may be affected in that region, which potentially includes the Bureau of Land Management's Las Cienegas National Riparian Conservation Area; however, a draft environmental impact statement (EIS) will likely be published in 2011. Based on the draft EIS, protective conservation measures may need to be implemented to protect the frog if the copper mine becomes developed. Mining actions taken without mitigating for the Chiricahua leopard frog's conservation may pose a notable threat to Chiricahua leopard frogs inhabiting the watershed shared by this proposed copper mine.

2.3.2.2 Overutilization for commercial, recreational, scientific, or educational purposes.

This is not considered an important threat to the Chiricahua leopard frog. No new information is available.

2.3.2.3 Disease and Predation

As illuminated by the threats assessment conducted during the preparation of the recovery plan, disease (chytridiomycosis) and predation by non-native species (e.g. bullfrogs, crayfish, fish, and tiger salamanders) are the most important threats to the Chiricahua leopard frog.

Disease: Chytridiomycosis

In some areas, Chiricahua leopard frog populations are known to be seriously affected by chytridiomycosis. As previously stated, chytridiomycosis is an introduced fungal skin disease caused by the organism *Batrachochytrium dendrobatidis* or "*Bd*". Voyles *et al*. (2009) hypothesized that *Bd* disrupts normal regulatory functioning of frog skin, and evidence suggests that electrolyte depletion and osmotic imbalance that occurs in amphibians with severe chytridiomycosis are sufficient to cause mortality. This disease has been associated with numerous population extirpations, particularly in recovery unit 6 in New Mexico, and with major die-offs in other populations of Chiricahua leopard frogs (USFWS 2007).

The frog appears to be less susceptible to mortality from the disease in warmer waters and at lower elevation. The precise temperature at which frogs can coexist with the disease is unknown and may depend on a variety of factors; however, at Cuchillo Negro Warm Springs, Sierra County, New Mexico, Chiricahua and plains leopard frogs (*Lithobates blairi*) become uncommon to non-existent where winter water temperatures drop below about 20 degrees Celsius (^{o}C) (68 degrees Fahrenheit (^{o}F)) (Christman 2006). A pH of greater than 8 during at least part of the year may also limit the ability of the disease to be an effective pathogen (USFWS 2007). Furthermore, based on experience in Arizona, particularly the Huachuca Mountains, if Chiricahua leopard frogs are absent for a period of months or years, the disease organism may drop out of the system or become scarce enough that frogs can persist again if reestablished. Essential breeding habitats either lack chytridiomycosis or include conditions that allow for persistence of Chiricahua leopard frogs with the disease as in warmer waters or at lower elevations.

The recovery plan discussed some research avenues for dealing with the fungal skin disease, chytridiomycosis, including use of anti-microbial peptides or cutaneous bacteria that have been found on the skin of amphibians, which can effectively combat *Bd*, the organism that causes the disease (Rollins-Smith and Conlon 2004, Harris *et al.* 2006). Retallick *et al.* (2004) found evidence that some populations of frogs apparently develop resistance to chytridiomycosis, and or the pathogen has developed less virulent strains that do not drive the host to extinction. Mendelson III *et al.* (2006) proposed using populations of frogs that showed some resistance to the disease as sources for reestablishments.

Building upon these hypotheses, we are attempting to find a disease-resistant population of another rare ranid frog – the Tarahumara frog (*Lithobates tarahumarae*), a species that disappeared from the United States in 1983, apparently due at least in part to chytridiomycosis. Efforts to reestablish the species in the U.S. have been thwarted by the disease. In 2008, we collected animals from a population in Sonora, Mexico, that has persisted with chytridiomycosis for more than two decades. We will use these animals as stock for future reestablishments, which will provide a test as to whether populations vary in their ability to persist with the disease in Arizona. We hope the results will inform decisions about Chiricahua leopard frog recovery, because, similar to the Tarahumara frog, some populations of Chiricahua leopard frogs have persisted with the disease, while others quickly perish. In addition to searching for frogs that can persist with the disease, and as described above in section 2.3.4, we have funded a study with USGS to examine the feasibility of using bacterial cultures, naturally occurring on amphibian skin, as a means to combat the disease. It is our hope that this 'probiotic' approach, proposed by Harris *et al.* (2006, 2009), may illuminate additional novel management tools for enhancing the persistence of Chiricahua leopard frog populations where chytridiomycosis is present. Until an effective solution is implemented, chytridiomycosis remains a serious threat to the Chiricahua leopard frog.

<u>Predation: Introduced aquatic species</u>

Introduced American bullfrogs, crayfish, salamanders, and fish species are known to be highly predaceous and are believed to negatively impact the Chiricahua leopard frog. Witte *et al.* (2008) found that sites with disappearances of Chiricahua leopard frogs were 2.6 times more likely to have introduced crayfish than were control sites. Unfortunately, few sites with bullfrogs were included in the Witte *et al.* (2008) and at many sites, there was no identification of the species of fish present.

Prior to the invasion of perennial waters by predatory, non-native species (American bullfrog, crayfish, fish species), the frog was historically found in a variety of aquatic habitat types. Today, leopard frogs in the Southwest, are so strongly impacted by harmful non-native species, which are most prevalent in perennial waters, that their occupied niche is increasingly restricted to the uncommon environments that do not contain these non-native predators, and these now tend to be ephemeral and unpredictable. This increasingly narrow realized niche is a primary reason for the threatened status of the Chiricahua leopard frog.

When the recovery plan was prepared, there was limited experience with predator control conducted over large areas. Recently, bullfrogs were eliminated from an approximately 5-mile radius of Peña Blanca Lake in recovery unit 1 and within 5 miles of Scotia Canyon in recovery unit 2. The former appears to be successful and the latter is showing success at eliminating bullfrogs from these areas. In addition, Chiricahua Leopard frogs recolonized Peña Blanca Lake and several stock ponds within the 5-mile radius of the lake within 6 months of eliminating bullfrogs from these locations. Coupled with previous work in the Altar Valley of recovery unit 1, the feasibility of bullfrog control is showing promise, at least in the short term. These projects inject some optimism into our capability for dealing with non-native predators on a landscape scale.

2.3.2.4 Inadequacy of Existing Regulatory Mechanisms

Non-native species used for fishing baits in Chiricahua leopard frog habitats present a vehicle for the distribution of these often predatory or competitive bait species into frog habitat and for the dissemination of deadly diseases to the frog, posing a significant threat. Picco and Collins (2008) found waterdogs (tiger salamanders; *Ambystoma tigrinum*) infected with *Bd* in Arizona bait shops, and waterdogs infected with ranavirus in Arizona, New Mexico, and Colorado bait shops. Furthermore, they found that 26–67 percent of anglers released tiger salamanders bought as bait into the waters where they fish, and 4 percent of bait shops released tiger salamanders back into the wild after they were housed in shops with infected animals, despite the fact that release of live salamanders is prohibited by Arizona Game and Fish Commission Orders. This study clearly showed the inadequacy of current State regulations in regard to preventing the spread of amphibian diseases via the waterdog bait trade. Additional regulation and/or increased enforcement of existing regulations are needed to stop the spread of amphibian diseases via use of waterdogs for bait.

2.3.2.5 Other Natural or Manmade Factors Affecting its Continued Existence

Small population size

Among the potential threats in this category discussed in the Chiricahua leopard frog recovery plan (USFWS 2007) and the final rule (67 FR 40790; June 13, 2002), are genetic and stochastic effects. These effects manifest in small populations, which can be related to genetic and demographic limitations, as well as environmental events and random catastrophies. Specifically, small populations are vulnerable to extirpation due to random variations in age structure and sex ratios, as well as from disease or other natural events that a larger population is more likely to survive. Inbreeding depression and loss of genetic diversity in small populations can also reduce the fitness of individuals and the ability of a population to adapt to change. The recent genetic study (Herrmann *et al.* 2009) revealed no systemic lack of genetic diversity within the Chiricahua leopard frog. In fact, populations were quite variable (up to 16 different genetic groupings were found). This does not preclude the possibility that individual populations may suffer from genetic or demographic problems, particularly if only a few individuals are available as mates or potential mates cannot be accessed due to their sparse distributions.

<u>Climate Change</u>

The Chiricahua Leopard Frog Recovery Plan (USFWS 2007) describes anticipated effects of climate change on the Chiricahua leopard frog. The plan cited literature indicating that temperatures rose in the 20^{th} century and warming is predicted to continue. Climate models are less certain about predicted trends in precipitation, but the southwestern U.S. is expected to become drier.

Since the recovery plan was prepared, the Intergovernmental Panel on Climate Change (IPCC) published a report (2007), which corroborates other pertinent literature (e.g. Seager *et al.* 2007, van Mantgem *et al.* 2009) that global warming is occurring and precipitation patterns are being affected. Also, the U. S. Environmental Protection Agency (EPA) published a “Proposed Endangerment and Cause or Contribute Findings” for greenhouse gases under section 202(a) of the Clean Water Act in April 2009 (EPA 2009). The document summarizes the recent literature on climate change and concludes that greenhouse gases are at unprecedented levels and that these levels are the root cause for climate change. Global mean surface temperatures have risen by 0.74^0 C (1.3^0 F) over the last 100 years and 8 of the 10 warmest years on record have occurred since 2001. Arctic ice caps are melting, sea levels are rising, rainfall patterns are shifting, the power and frequency of Atlantic hurricanes are increasing, and other symptoms of climate change are documented in the EPA document. The EPA (2009) predicts that warming in the 21^{st} century will exceed what occurred in the 20^{th} century, even under scenarios of low emissions growth.

According to the IPCC report, global mean precipitation is anticipated to increase, but not uniformly (IPCC 2007). In the American Southwest and elsewhere in the middle latitudes, precipitation is expected to decrease. Drought in the western U.S. is expected to increase, snow packs will decline, and snowmelt will occur earlier (EPA 2009). Although most climate models predict a drying trend in the 21^{st} century in the southwestern U.S., these predictions are less certain than predicted warming trends. The models do not predict summer precipitation well, and typically at least half of precipitation within the range of the Chiricahua leopard frog occurs in the summer months (Brown 1982, Guido 2008). Furthermore, there have been no trends in summer rainfall over the last 100 years in Arizona (Guido 2008), nor since 1955 in annual precipitation in the western

United States (van Mantgem *et al.* 2009). On the other hand, all severe, multi-year droughts in the southwestern U.S. and northwestern Mexico have been associated with La Niña events (Seager *et al.* 2007), during which sea surface temperatures in the tropical Pacific decline. Climate models predict that drought driven by La Niña events will be deeper and more profound than any during the last several hundred years (Seager *et al.* 2007).

Drought has likely contributed to loss of populations since the species was originally listed. Stock tank populations are particularly vulnerable to loss, because they tend to dry out during periods of below normal precipitation. These trends are likely to continue, but the situation is complicated by interactions with other factors. For example, the effects of drought cannot be separated from the effects of introduced aquatic predators, because drought will affect those predators as well as populations of Chiricahua leopard frogs. The interaction between predators and drought resistance of frog habitats is often a delicate balance. Stock tanks are likely an important habitat for leopard frogs in part because these sites dry out periodically, which rids them of most aquatic predators. Leopard frogs can often withstand drying of stock tanks for 30 days or more, whereas fish and bullfrogs may not. However, if stock tanks dry for longer periods of time, neither leopard frogs nor introduced predators may be capable of persisting. Drought will reduce habitats of both leopard frogs and introduced predators, but exactly how that will affect recovery potential for the Chiricahua leopard frog will probably be site-specific; the effect on recovery potential overall is not yet clear.

Additionally, temperature appears to influence the impact of chytridiomycosis to the frog, and temperature may also impact introduced predator populations. Sites where Chiricahua leopard frogs coexist with the disease are typically at lower elevations and are warmer sites (USFWS 2007). As a result, if temperatures increase as predicted, perhaps more populations will be able to persist with the disease. Furthermore, the disease is spread in part by introduced bullfrogs and tiger salamanders (USFWS 2007), hence the effects of the disease are intertwined with trends in introduced predator populations and their ability to tolerate climate changes and disperse across the landscape. Although bullfrogs, like Chiricahua leopard frogs, are tolerant of warm water temperatures, the comparative survivability of each species across a range of temperatures for periods of time is unknown (Palenske and Saunders 2003). Under increasing temperatures, habitats between aquatic sites could become harsher and less conducive to dispersal, which may limit the spread of bullfrog or salamander populations and benefit the Chiricahua leopard frog by reducing the spread of predation and disease. However, warmer, drier conditions would likely shrink aquatic habitats and possibly concentrate predator populations into more restricted areas, which could increase predation pressure and disease exposure for Chiricahua leopard frogs in shared aquatic sites. Overall, temperature and moisture changes could alter the roles of introduced bullfrogs, salamanders, and fishes as predators and vectors of chytridiomycosis, but the interactions and effects upon the Chiricahua leopard frog are not clearly understood. Thus climate change, particularly in the form of drought, but also in terms of water temperature variation, poses a significant threat to the Chiricahua leopard frog into the foreseeable future.

2.4 Synthesis

New information generated in the last few years improves and updates our understanding of the status, threats, and recovery potential of the Chiricahua leopard frog. Molecular genetics studies, employing both mtDNA and microsatellites from samples throughout the range of the species, indicate that *Lithobates chiricahuensis* is a single species, although with considerable variability and genetic structure. As a result, it is not appropriate at this time to consider listing separate DPSs, subspecies, or species within what is currently recognized as the Chiricahua leopard frog. That said, once the genetics study is finalized, the technical subgroup of the recovery team should consider how the genetic groupings correspond to recovery unit boundaries. Adjustment of those boundaries may be warranted, although presumed relatedness was only one factor in designing recovery units.

Evidence indicates that since the time of listing, the species has probably made at least modest population gains in Arizona, but is apparently declining in New Mexico. Overall in the U.S., the status of the Chiricahua leopard frog is either static or, more likely, improving, with much of the increase attributable to reintroduced individuals (mainly in Arizona) from captive rearing programs. Status and trends are unknown in Mexico. An aggressive recovery program is underway in the U.S. that is showing considerable results on the ground. The reestablishment of populations, creation of refugial populations, and enhancement and development of habitat have helped stabilize or improve the status of the species in some areas. The status of the Chiricahua leopard frog should be closely monitored throughout its range in accordance with the recovery plan. In particular, population and threats monitoring should be initiated in Sonora and Chihuahua, Mexico.

In the American Southwest, increasing temperatures and a drying trend, at least in the winter months, are anticipated symptoms of climate change driven by unprecedented levels of greenhouse gases. Precisely how the Chiricahua leopard frog and its habitat will be affected is unclear, in part because of interrelated and indirect effects revolving around the response of non-native predators and disease to climate change, the frog's two most significant threats at this time. In particular, persistence of Chiricahua leopard frogs infected with the fungal skin disease, chytridiomycosis, is enhanced in warmer waters, but introduced predators are often carriers of chytridiomycosis, and drought driven by climate change could limit their numbers or ability to move about on the landscape. Furthermore, some Chiricahua leopard frog sites are buffered from the effects of drought by wells or other anthropogenic water supplies.

In summary, there is no evidence that the Chiricahua leopard frog is declining across its range. And although full recovery is still distant in most recovery units, significant progress has been made to secure existing populations and establish new populations. If the range-wide status of the species declines in the future, endangered status should be considered. However, until such time, it is appropriate to leave the listing status unchanged.

3.0 RESULTS

3.1. Recommended Classification:

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

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

Brief Rationale: A 2C recovery priority number is indicative of a full species with a high degree of threat and a high potential for recovery, in conflict with construction, development projects, or other forms of economic activity, primarily in the form of water demands. Based on this review, threats to the Chiricahua leopard frog remain high, such as the disease chytridiomycosis, predation from introduced aquatic amphibians and fishes, and water diminishment due to human demand and drought, among others. Yet, the potential for recovery also remains high for this species, given its genetic diversity and potential for persisting in a variety of aquatic habitats, the demonstrated successes in Arizona with collaboration and management, the willingness of private landowners to provide high quality habitat, the potential of effectively eliminating non-native predators at local scales, and the possibilities of developing resistance to chytridiomycosis. Furthermore, because the species continues to decline in some areas due to water loss, the frog's need for perennial water remains in conflict with human needs for water in the arid Southwest.

3.3. Listing and Reclassification Priority Number: Not applicable.

4.0. RECOMMENDATIONS FOR FUTURE ACTIONS

There are six categories of actions needed to recover the Chiricahua leopard frog (see "Recovery Strategy" page 49 of the Chiricahua Leopard Frog Recovery Plan (2007)):

1) protect and manage remaining populations and habitats (recovery action 1);
2) restore and create habitat, and establish additional populations as needed to build viable metapopulations and isolated robust populations in each recovery unit (recovery actions 2, 3, and 4);
3) monitor progress towards recovery (recovery action 5);
4) research the conservation biology of the frog with the objective of facilitating efficient recovery (recovery action 6);
5) develop support and build partnerships to facilitate recovery (recovery actions 7-11); and
6) practice adaptive management in which the recovery plan and management actions are revised to reflect new information developed through research and monitoring (recovery action 12).

In 2009, a five-year plan outlining the most critical recovery needs for the Chiricahua leopard frog was completed (Spotlight Species Action Plan, U.S. Fish and Wildlife Service 2009). Critical actions included:

1) protect remaining populations and habitats (recovery action 1);
2) establish new populations in areas with minimal or manageable threats (recovery action 3);
3) develop a comprehensive monitoring plan (recovery action 5.1);
4) implement monitoring, particularly of extant populations and habitat conditions where the species occurs (recovery action 5.3);
5) begin researching techniques to ameliorate the threats of disease and non-native predators (recovery actions 6.13, 6.19);
6) work with partners, stakeholders, and technical experts to build support for the recovery effort and to facilitate implementation of recovery actions;
7) complete the range-wide genetics study (recovery action 6.14); and
8) find funding and staff time to support the recovery program (recovery action 7.1).

Currently the biggest obstacle to recovery is a lack of funding and staff time. We have willing partners and know how to establish populations and manage threats in some types of aquatic systems. With adequate funding and staffing, we could target sites with manageable levels and types of threats and move toward meeting the recovery criteria in most of the recovery units.

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U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW
Chiricahua leopard frog

Current Classification: Threatened

Recommendation resulting from the 5-Year Review:

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

Appropriate Listing/Reclassification Priority Number, if applicable: Not applicable

Review Conducted By: Jim Rorabaugh, Supervisory Fish and Wildlife Biologist, U.S. Fish and Wildlife Service, Arizona Ecological Services Office

FIELD OFFICE APPROVAL:

Lead Field Supervisor, Arizona Ecological Services Field Office, U.S. Fish and Wildlife Service

Approve ______________________ Date 12/17/10

Cooperating Field Supervisor, New Mexico Ecological Services Field Office, U.S. Fish and Wildlife Service

Approve ______________________ Date 12/17/10

REGIONAL OFFICE APPROVAL:

Assistant Regional Director, Ecological Services, U.S. Fish and Wildlife Service, Region 2

Acting

Approve ______________________ Date 1/28/11